PLANT PHYSIOLOGICAL CHEMISTRY

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PLANT PHYSIOLOGICAL CHEMISTRY

BY

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TO

MARY VANDERVORT HESTER HARVEY

MY MOTHER

"Je n'ai point eu d'autre livre, que le ciel et la terre, lequel est connu de tous, et est donné à tous de connoistre et lire ce beau livre."—Bernard Palissy (1510?–1589?).

PREFACE

It is the purpose of this text to present the physiological chemical mechanism of the vital processes of plants. Most attention will be given to the metabolic reactions, with some of the chemistry underlying the process, but complete description of the chemical compounds of plants does not lie within the province of this text. For the physical and chemical properties of plant constituents, the student should refer to texts of organic chemistry, especially such as Holleman-Walker, *Textbook of Organic Chemistry;* Perkin and Kipping, *Organic Chemistry;* Haas and Hill, *The Chemistry of Plant Products;* Leathes, *The Fats;* Osborne, *The Vegetable Proteins;* Jordan Lloyd, *Chemistry of the Proteins;* H. E. Armstrong, *The Simple Carbohydrates and Glucosides;* or to Fr. Czapek, *Biochemie der Pflanzen.* For exhaustive discussions of photosynthesis, reference should be made to the monographs on *Photosynthesis* by H. A. Spoehr and by W. Stiles. There is an excellent monograph on *Plant Respiration* by S. Kostytchev, edited by Lyon.

Free use has been made of every source of information for the compilation of this text. It is considered that profuse citation of the authors leads to the confusion of students. Sufficient indices are now available so that any one interested in compiling a bibliography of a particular subject can do so with facility. No great amount of new research data is presented here, but the material which has been used for illustration is of a nature such as should give a new point of view to students of plant physiology and of the plant sciences in general. The main part of the discussion is devoted to the synthesis, translocation, storage, digestion, and utilization of substances within the plant. The viewpoint is mainly physiological, but the interpretation necessarily involves chemistry and physics. It is assumed that the student has some knowledge of chemistry and physics as well as of general botany and plant physiology.

It is taken by the author that there is much needed a summary of the information on the chemical mechanism in plant physiology. The choice of material to be presented to the student as illustrative of the plant chemical mechanism is purely a matter of opinion. To express an opinion at all is treading upon the borders of omniscience and is certainly dangerous to personal reputation; yet to have progress we must have definite hypotheses. It is considered more desirable to state a definite hypothesis although it may not fit every condition and may not in the future be proved true, than to confuse the student with every angle of

argument presented in the literature. For complete statement of the literature of many of the topics here presented the student should refer to Czapek's *Biochemie der Pflanzen* and to more recent literature.

The author is indebted to many sources for photographs and drawings, and for the use of data and translations. At the request of Sir E. J. Russell, a portrait of Dr. Woodward was kindly taken by the British Museum staff from their collection of prints. The Direction of the Deutsches Museum, Munich, gave permission to use a portrait of Van Helmont from the memorial window in the Alchemists' Laboratory. Copies of portraits of several physiologists were supplied by Dr. John W. Harshberger of the University of Pennsylvania. Mr. J. C. Bay, Librarian of the John Crerar Library, Chicago, has made available many rare books and prints from his collection.

Valuable criticisms on particular chapters have been extended by Dr. A. J. Pieters, who read the part on General Metabolism; Mr. George Nesom, who read the parts dealing with Mineral Nutrition; Dr. Charles Shull, who made suggestions for the chapter on Fats; Dr. W. A. Gardner, who read Proteins; Dr. A. L. Bakke, who criticized the part on Photosynthesis; and Dr. R. P. Hibbard, who read the part on Respiration.

To these men I am especially indebted for suggestions and criticisms; yet they are in no way responsible for errors or arrangements.

My wife, Helen M. Whittier Harvey, has corrected the manuscript, assisted with arrangement and drawings, and compiled the index. Miss Gladys Anderson of the Division of Plant Pathology and Botany has assisted with the manuscript and illustrations.

It is hoped that the publication of so meager a text as this will stimulate to activity those better qualified, whose writings in English have been so long delayed and are so much needed.

The greater part of the manuscript was written while the author was on sabbatical leave from the University of Minnesota as a Fellow of the John Simon Guggenheim Memorial Foundation, at Cambridge University.

RODNEY BEECHER HARVEY.

University of Minnesota,
April 18, 1929.

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INTRODUCTION

MECHANISM OF THE TRANSFORMATION OF MATERIALS IN PLANTS

I. *Effect of Protoplasmic Phases on Chemical Reactions of the Cell*

The protoplasm of the plant cell forms the medium in which the life processes are carried on. Protoplasm in actively metabolizing cells is mostly water; hence the transformations must take place in this basic substance of the cell. The water is distributed through a series of heterogeneous phases which form the plastids, the chromosomes, and all other organs of the cell. Some phases in the cell are rich in water and must be considered as simply aqueous solutions of substances. The vacuolar sap is mainly a simple solution of water-soluble substances, and in some cells the vacuole makes up the greater part of the cell volume. In highly vacuolated cells the sap may contain little or no colloidal material. The rest of the cytoplasm is relatively rich in colloids, both in aqueous phases made up of proteins and carbohydrates, and non-aqueous phases of fats and lipoids. These aqueous and non-aqueous phases exist side by side in the most intimate mixture. Owing to the presence of phosphatides which imbibe water and are soluble in oils, water may be present in both the aqueous and lipoid phases. Shifting of water from phases rich in water to those containing little of it may greatly affect the rate of such chemical transformations as hydrolyses. There may be reversal of phases in the cell; water, the more important external phase, may become the internal phase, and the oil phase, rich in phosphatide, may become external. In fact, this phase inversion has been hypothecated as of considerable importance in determining the entrance of substances into the cell, whether lipoid-soluble substances through lipoid phases, or water-soluble substances through aqueous phases. Evidently there is a delicate balance in this critical condition in the live cell. Anything which tends to throw the system of phases considerably out of the equilibrium condition may change the cell permeability or cause death. A balance of phases must be maintained to make possible all of the vital reactions.

The largest masses of single phases in the cell are the cell vacuoles. These may make up practically all of the cell volume in old cells. Young cells are much richer in colloidal materials than old cells.

While the cell is alive, there are continually occurring processes which tend to keep the phases in the cytoplasm in a finely divided state. The

phases are continually peptized to highly colloidal particles. If any agency tends to increase unduly the size of the particles, the vital activities may cease. Flocculation or precipitation of the proteins may result in death. This continual peptization of the protoplasm is evidence that the surface tension between the phases is relatively low, because the phases are easily distributed in each other when the interfacial tension is low.

A high state of division of the phases of the protoplasm is necessary for rapid chemical transformations. Increasing the division of a sphere of oil 1 mm. in diameter into globules 1 μ in diameter increases the surface a million times. When large surfaces of phases are exposed, the reaction may proceed rapidly. The emulsification of oil into droplets of colloidal dimensions leads to quick digestion. This dispersal of an oil phase in the cell is accomplished by decreasing the surface tension at the interface between oil and water phases. This may be accomplished by emulsifying agents such as phosphatides or by means of soaps, which are formed through the action of ions from the aqueous phases upon the surface of the oil droplet.

The amœboid movements of the cell are dependent upon changes in surface tension produced either by changes in the materials forming the surface layer or by changes in the electrical charge across the interface. The products of metabolism may be used to bring about these changes. For instance, urea produced from the breaking down of protein may change the surface tension at the cell interfaces. Anesthetics also may change the interfacial tension and thus affect cell permeability or the exchange of substances between phases.

II. *Interactions between Phases of the Protoplasm*

The distribution of substances between phases is determined by their relative solubilities in each phase. The partition coefficient is the percentage distribution of a substance between two phases. Thus between phases of water and of chloroform, iodine will be distributed in proportion to its relative solubility in these two substances. Iodine is almost insoluble in water, so the greater amount of iodine will be found in the chloroform layer. The partition coefficient, $\frac{\text{chloroform}}{\text{water}}$ is high numerically. But if there is a chemical change in the distributed substance in one of the phases, there will be a redistribution of the substance which may markedly change the partition between the phases. Thus, if to the aqueous phase in the system, iodine distributed between water and chloroform, there is added a small amount of potassium iodide, a complex ion will be formed between iodine and KI in the aqueous phase. Then most of the iodine will diffuse from the chloroform and form this

complex ion in the aqueous phase. The iodine will migrate from one phase to the other, owing to the introduction of the KI with which it forms a complex ion. In a similar manner, substances in the cell may be caused to migrate from one phase to another, and such changes in the concentration of a substance may affect markedly the metabolic reactions.

Any substance which decreases the interfacial tension will tend to accumulate at the surface. This is for the reason that the free energy of the system is decreased by the decrease in interfacial tension. The resultant increase of the concentration of a substance between phases may bring to position for transformation or interaction the substances contained in the various phases of the cell and thus facilitate chemical interactions by increasing the concentrations of the substances in condition to act. Since the interfacial tension is subject to fluctuation and probably is regulated by the cell, the cell may initiate or decrease certain reactions by this means. The cell is organized and correlated in the functions of its parts and is not merely passive and at the mercy of the environment. In fact, one of the commonest traits of organisms is to react against their environment.

III. *Rate of Chemical Reactions*

The rate of a chemical transformation is determined by the concentrations of the molecules in condition to react. When the energy concerned in a reaction is very great, the position of the equilibrium between the reacting substances will be such that as great as possible an amount of energy will be evolved. That is, the reaction will go practically to completion in the direction in which the energy is released. But when the energy liberated is small, the position of equilibrium will be such that the reaction does not go to completion and there will be found appreciable concentrations of the reacting substances and the products of their interaction.

If any component of the reacting system is withdrawn into another phase, then reactions involving small energy transfers may proceed more nearly to completion, because the substance in passing into another phase is not in condition to act, and its mass action effect is decreased. This condition is of much importance in so heterogeneous a system of phases as the protoplasm. Substances are stored in the cell by being withdrawn into solid phases, as into crystals of proteins, or insoluble starch, or into oil globules. At the interface between phases the atomic groups of molecules in the phases are oriented. The atomic groups are placed so that those groups with water affinities are in aqueous phases and those with lipoid affinities are in oil phases. Orientation at the surface may bring groups into position to react.

The simplest case of chemical transformation is one involving the decomposition of a substance. In such a decomposition the rate of the reaction or the amount of decomposition products produced in unit time is dependent upon the concentration of molecules of a single substance. Such chemical reactions are called mono-molecular, or reactions of the first order, and they proceed at rates which are directly proportional to the concentration of the substance undergoing decomposition.

When the chemical reaction is dependent upon the interaction of two different substances, the rate will be dependent upon the product of the concentrations of both of these substances which are in condition to act. The transformation will proceed at a rate which is directly proportional to the product of the molar concentrations of the two reacting substances. Such reactions are called bimolecular, or reactions of the second order. When one substance is greatly in excess, its concentration during the reaction may not change appreciably, so that the reaction will proceed almost as if it were dependent upon the concentration of a single substance, that is, practically as if it were a reaction of the first order.

When chemical reactions are dependent upon the meeting of three or more molecules at the same time, the opportunity for the reaction to occur is greatly decreased. The chances of meeting are directly proportional to the product of the concentrations of the three substances, and the rate of the reaction will be directly proportional to this product.

Reactions of the first order, such as simple molecular decompositions, may proceed at a very high rate. Reactions of the higher orders decrease in rate very rapidly in proportion to the number of the substances required for the reaction. In fact, in many reactions involving several molecules, the course of the reaction consists of a series of reactions between two or more substances to produce an intermediate product which then may further react to give the final product. The controlling reactions of many vital processes may be treated as systems of irreversible first order reactions. This allows the reaction of a high order to proceed at the rate of a series of reactions of the lower orders.

Several theories have been advanced to account for the initiation of chemical reactions. According to the intermediate compound theory, there is concerned a series of reactions requiring the formation of unstable intermediate compounds. The intermediates in some reactions may be isolated and identified; in others they may be merely hypothecated. This intermediate compound hypothesis is used especially to explain complex reactions. Intermediate compounds are of much importance in determining the rate of reactions involving several substances. Introduction of such intermediates into the reacting system may greatly increase the rate of the reaction. A reaction may be pro-

ceeding at so slow a rate as to be imperceptible. The introduction of intermediate compounds may then be said to initiate the reaction.

In the chemical transformation theory it is assumed that in the molecule the atoms or atomic groups have cyclic motions perhaps with different critical periods of oscillation. In one particular position of the atomic arrangement the molecular system is unstable and atomic groups may rearrange themselves and not return to their path of oscillation but react to form more stable systems. Only a certain part of the molecules may have this critical arrangement at one time, that is, only a part of the molecules are in a reactive condition. Such might be the case in molecular decompositions such as in the decomposition of $2H_2O_2$ into $2H_2O + O_2$. Such substances should show spontaneous decomposition when no energy need be supplied from without.

According to the kinetic theory of molecular activation, a certain number of molecules in a system at any instant possesses a greater velocity of translation than the average kinetic energy of the molecules in the system. The kinetic energy can be converted to increase the amplitude of the oscillation of atomic groups. The greater energy of these "hot" molecules may come from the accident of collision with others. When the value of the kinetic energy exceeds a certain limit, the amplitude of oscillation of atomic groups in the molecule may become great enough to cause a decomposition or rearrangement in the molecular structure. The rate of the chemical transformation then is determined by the rate at which the velocities of the molecules are accelerated beyond the critical limiting value of kinetic energy. The effect of a rise of temperature in increasing the rate of such reactions is due to increasing the average kinetic energy of the molecules in the system.

It has been found that the velocity of chemical reactions is doubled or trebled by each rise of 10° C. This effect is known as the temperature coefficient or Van't Hoff coefficient of the chemical reaction and is designated by the symbol Q/10. The value of the temperature coefficient for a process is useful because it indicates the nature of the factors which determine the rate. When physical factors such as diffusion, etc., are concerned, the temperature coefficient is of the order of Q/10 = 1 to 1.5. However, the value of Q/10 is constant only for a certain temperature range.

A more accurate formulation of the effect of temperature on the velocity of irreversible reactions is the following:

$$\frac{\quad}{K_0} = e^{\angle \quad T_1 T_0}$$

in which K_0 and K_1 are the velocity constants at the respective absolute temperatures T_0 and T_1. μ is the energy of activation. μ is found by

dividing the gram molecular energy of activation E of the active substance by RT. R is the gas constant, and T is the absolute temperature. When the reaction is of the second order, the sum of the energies of activation of the two active substances is taken. The velocity of the reaction is proportional to the exponential of $\frac{E}{RT}$.

The constant μ, known as the temperature characteristic, designates the energy of activation of the process. When the same species of active molecule is concerned, the value of the constant μ will be the same for various reactions. Various reactions determined by the same catalyst then should yield a practically constant value of μ. Fig. 1. The critical thermal increment is independent of the amount of the catalyst. An abrupt change in the value of μ over the temperature range of a process indicates that the nature of the active substance has changed. Thus the controlling reactions of a process may be the catenary series $\underset{K_1}{O \longrightarrow} \underset{K_2}{A \longrightarrow} E$, in which the original substance O is changed into an available form A, and this is decomposed to the end products E, with liberation of energy. The velocity constants of the two reactions may be K_1 and K_2. The effect of temperature rise on the two stages in the process may be different, so that different values of μ are obtained, depending on whether the rate of the process is determined by O→A or A→E, which may be catalyzed by different agents. Different temperature coefficients are shown by the same chemical reaction when it is catalyzed by different catalysts. The critical thermal increment may be used to indicate the catalyst of a reaction.

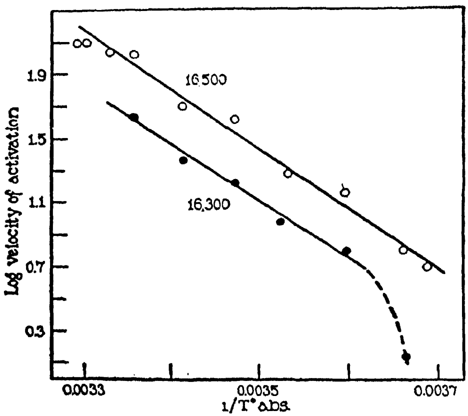


FIG. 1.—The velocities of the activation process occupying the "presentation time" for geotropic response ($\mu = 16,300$) and for phototropic response ($\mu = 16,500$) in *Avena*. These processes are controlled by a basic reaction which is probably a catalyzed respiratory oxidation. (After Crozier.) From *J. Gen. Phys.*

The critical thermal increment for most chemical processes is above 10,000. When such physical factors as diffusion or surface action are concerned, the value may be lower, near 7,500.

IV. *Mechanism of a Reaction*

In the chemical transformations in cells, all of the conditions given above may be effective. It is of interest to follow through a series of complex changes such as have been supposed to occur in the hydrolysis of sucrose by H^+.

The actual reactants are supposed to be sucrose dihydrate and H^+. The sucrose dihydrate has two molecules of H_2O attached to the d-glucose part; the fructose part is anhydrous. The sucrose dihydrate and H^+ combine at a measurable speed, giving a complex which immediately reacts with H_2O to give one molecule of d-glucose and one of d-fructose.

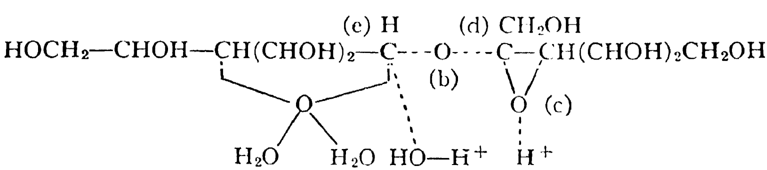
$$C_{12}H_{22}O_{11}2H_2O + H^+ \underset{\text{measurable speed}}{\rightarrow} (C_{12}H_{22}O_{11}2H_2OH + H^+)$$

$$(C_{12}H_{22}O_{11}2H_2OH^+) + yH_2O \rightarrow \text{d-glucose } 2H_2O + \text{d-fructose} + (y-1)H_2O$$

immeasurably fast

Only about 35 calories are involved in breaking the oxygen linkage ring of sucrose. This is a relatively small amount of energy; hence the reaction is easily reversible, and heat may be absorbed from the environment. In reactions in which great energy changes are involved, the reversibility is not so easy; shorter wave-lengths of light may be required as a source of the energy.

It is probable that the fructose part of the sucrose molecule is the reactive part. Several lines of evidence indicate this. First, hydroxyl ion will not invert sucrose. Also, fructosides in general are easily hydrolyzed, owing to the reactivity of the fructose part of the molecules. Finally, hydrogen ion is specific in the inversion. The rate of formation of the H^+ compound with sucrose determines the speed of the reaction. This gives the reason for the specificity of H^+ in the inversion. The reaction may be represented as follows:



In the initial formation of the sucrose hydrogen ion complex a partial valence of the oxygen atom (c) is used up, in consequence of which the end carbon atom (e) of the glucose portion effectively gains a free positive valence. This immediately links up with the hydroxyl of a neighboring

water molecule to give a system which may be represented as in the figure. There is an ethylene oxide ring which is normally very unstable in the molecule with a weak linkage at the oxygen atom (c). The ring therefore breaks at the dotted line C - - - - O, leaving a free valence at the carbon atom (d). Thus a new CHOH grouping is temporarily formed in the fructose residue. It is obvious that this could not have happened had any ion other than H^+ been attached to the sucrose in the first place. The carbon atom (d) now has a free valence which can so strengthen the carbon-oxygen linkage between (d) and (b) that the already weakened linkage between (e) and (b) would be completely broken. As a result of the free valence produced at (e) its union with the OH is now strengthened with the result that an H^+ different from the original is split off.

Further evidences of the conditions affecting the rate of inversion of sucrose by H^+ can be gained from the Law of Mass Action. The Law of Mass Action states that if the temperature is held constant, the velocity of a chemical reaction is directly proportional to the product of the concentrations of the substances in condition to react. The rate of sucrose inversion then is proportional to the product of the concentrations of sucrose, water, and H^+. The reaction velocity is not equal to the product of the concentrations but directly proportional to it. To make a proportionality into an equality we must introduce a constant, usually designated as k, into the proportionality.

If a reactive substance is very greatly in excess, its concentration can be taken as unchanging or as a constant. In a dilute solution, water may be taken in great excess and the H^+ may be held constant. Then the rate of the reaction will vary proportionally with the concentration of one substance only, and the reaction will proceed practically as a monomolecular reaction. But in a concentrated sugar solution the concentration of water may not be taken as constant, and the reaction proceeds as a bimolecular reaction.

Suppose one-half of the substance in a monomolecular reaction was found to disappear in ten minutes. If the concentration of the substance were diluted to one-half, then one-half of the original amount would disappear in ten minutes. Accordingly, by doubling the original concentration, the quantity of substance cleaved would be doubled. The inversion of cane-sugar proceeds practically as a monomolecular reaction if dilute solutions are used, so that the water is greatly in excess.

If dx represents the small amount of cane-sugar which disappears in the small time interval (dt), then $\frac{dx}{dt}$ is the velocity of the inversion reaction.

Let a represent the amount of cane-sugar present at start.
Let x represent the amount of cane-sugar which disappears in time (t).
Then $a-x$ = concentration at the end of time (t), and the velocity immediately after this time is proportional to $a-x$.

Then $\frac{dx}{dt} = k(a-x)$.

We wish to find the value of k, the constant of the reaction.
Integrating, $-\ln(a-x) = kt + \text{constant}$.

If we take the conditions at the start of the reaction when no time has elapsed (hence $t=0$) no sugar has been inverted (hence $x=0$). Then substituting these values,
$-\ln(a-0) = KO + \text{constant}$ or $-\ln a = \text{constant}$.

Substituting this value of the constant:
$-\ln(a-x) = kt - \ln a$ or $kt = \ln a - \ln(a-x)$ or $k = \frac{1}{t} \cdot \ln\frac{a}{a-x}$.
Briggsian logarithms (log) multiplied by 2.3025 give the natural logarithm (ln); therefore we may write $k = \frac{1}{t} \cdot 2.3025 \cdot \log\frac{a}{a-x}$.

The number 2.3025 may be included in k, the constant of the reaction,

so, $k = \frac{-}{t} \cdot \log \frac{}{a-x}$

The concentration of sucrose can be determined by reading the rotation in degrees given by the polarimeter. The change of this value during inversion gives stages in the reaction. If we measure the time to produce a certain change of rotation, we can determine the rate of the change, that is, the velocity of the reaction, or the value of k.

The following experimental data on the inversion of sucrose may be used in calculating the value of the reaction constant, k:

T. in minutes	*Rotation*	$k = \frac{1}{t} \cdot \log \frac{a}{a-x}$
0	46.76	
45	38.25	.001344
90	30.75	.001352
150	22.00	.001321
210	15.00	.001371
270	8.25	.001425
390	−1.75	.001499
510	−7.00	.001463
630	−10.80	.001386
	−18.70	

The value of k is practically uniform; hence it appears true that the reaction proceeds essentially as a monomolecular reaction. If the concentration of water is not great, then the change in concentration of this component of the system may not be disregarded, and the reaction speed is not that of a monomolecular reaction, and k will show a trend in its values if it is calculated from the formula for a monomolecular reaction. It will be found in the inversion of sucrose in plants that the quantity of free water may be small, so that the water concentration may affect the rate of cleavage of sucrose.

In the laboratory, if we determine the value of k at different hydrogen ion concentrations, we may plot these values against pH and thus determine the relation of k, the constant of the reaction, to pH, or the rate of inversion as determined by H^+ concentration.

The rate of inversion of sucrose in acid solutions has been found to be directly proportional to the H^+. Hence fluctuations in the pH of the cell sap will produce corresponding fluctuations in the rate of cleavage of sucrose.

V. *Catalytic Action*

Chemical transformations may be increased or decreased in rate by the presence of chemical substances and by certain physical agents. If a substance by its presence alone increases the rate of a reaction, it is called a positive catalyst; if the rate of the reaction is decreased, the substance is called a negative catalyst.

A true catalyst is not consumed in the reaction. It may act in several ways. First, by its physical effect of causing the adsorption of the reacting substances upon its surface. For example, platinum black is a positive catalyst in the reaction between H_2 and O_2. One or both of the reacting gases may be adsorbed on the surface of the platinum, and the concentration so increased as to initiate the combination. Since great quantities of energy are involved in this reaction, it may proceed at an explosive rate when once initiated by the introduction of platinum black into a vessel containing H_2 and O_2.

Second, the substance introduced may act as an intermediate substance, increasing the rate of the reaction in proportion to its concentration, or it may form an intermediate substance by interaction with one of the components of the system. Thus the introduction of phosphate into a mixture of zymase and glucose increases the rate of the production of alcohol and CO_2, because phosphate combines with the sugar to form hexose phosphate, an intermediate compound in this reaction. The substance introduced may combine with intermediate compounds already present in the reacting mixture, decomposing or removing the inter-

mediate, in which case the introduction of the substance may decrease the rate of the reaction, or it may act as a negative catalyst.

In reactions which require the introduction of energy, heat or light may catalyze the reaction simply by supplying the energy required. If the energy required to be stored in the products is small, heat may be absorbed from the environment and stored in the products of the reaction. The effect of heat as a purely catalytic agent may be such as to increase the average kinetic energy of the molecules, thus increasing the number of collisions in unit time; but frequently such kinetic energy or a part of it may be stored in the reaction products by the mechanism previously outlined. For such reactions a rise of temperature should increase the rate of the reaction.

When the energy required for the reaction is great, heat may not serve as the source of energy, but shorter wave-lengths of light may be required. On the basis of the theory that radiant energy is absorbed in definite units or quanta, the size of the quantum varies inversely with the wave-length, that is, the shorter the wave-length is, the greater is the quantum of energy involved. Perhaps the difference in action of different wave-lengths is caused by the ability of energy to be absorbed only when the quantum is of the exact size required to supply the energy needed for the rearrangement of the atomic groups. When only small amounts of energy are involved, heat at low vibration frequency can supply the proper sized quanta, while the rapid vibrations of light are required to coincide with the quanta concerned in reactions involving great energy changes. Only light which is absorbed can produce chemical change. The absorption of light is dependent upon the presence of atomic groups or electrons in the substance which can take on quanta of the exact size contained in the radiant energy.

The immediate effect of the absorption of a light quantum is the formation of an activated or "hot" molecule. The energy of the electronic system of such molecules is increased. The binding forces between atoms may be weakened by this change of electronic energy, and the molecule may undergo dissociation if the absorbed energy quantum is great enough.

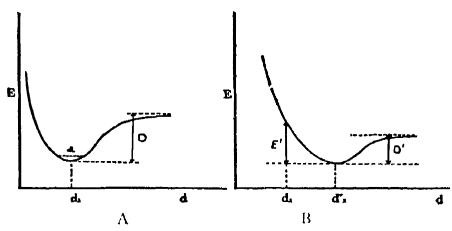


FIG. 2.—For explanation see text. (After Kistiakovski.)

The intramolecular processes following light absorption can be best illustrated by the following diagrams (Fig. 2) on which the potential

energy of atomic oscillations E is represented as a function of the relative distance of the atoms d. Figure 2A represents the state of a normal molecule prior to light absorption, the atoms oscillating with small amplitude (a) around the equilibrium position d_1. The change of electronic configuration on absorption of a light energy quantum, resulting in a change of the binding forces, will increase the equilibrium distance (to d'_1, see Fig. 2B) in case the binding force has been weakened. During this process the atoms will have (on account of their slow motion) no opportunity to change their position and will be still at an average distance d_1. Their potential energy therefore will be increased (by E') and, provided this energy increase is larger than the energy of binding of the excited molecule (D'), the atoms will fly apart; that is, a dissociation will take place, within a time interval comparable to the time of one oscillation.

If no energy is absorbed the reactions of single atoms and molecules occur only when they are accompanied by a decrease in the energy of the reacting system. The quantum energy of absorbed radiation must be larger than the heat of dissociation of the reacting molecules. The quantum energy being smaller, no dissociation will take place and activated molecules must be formed. These can react only on collisions and only in such a way that the heat absorbed in the resulting chemical change is smaller than the quantum of radiation absorbed. The molecule may be raised to a higher quantum state by the absorption of a light quantum, forming an activated molecule. Molecules may remain undissociated after the absorption of a quantum greater than that required for dissociation. These activated molecules possess a high reaction ability and will undergo chemical change on collision with some suitable non-activated molecule in the reacting system. The excited molecules may reëmit energy quanta, either heat or light, usually of lower vibration frequency than the light absorbed. The excited molecules also may lose the energy stored in activation by inelastic collisions with inert gas molecules.

Diatomic and polyatomic molecules possess a large number of quantum levels, and the loss of excitation energy will occur generally in small steps. In this way the absorbed light energy will gradually be dissipated and transformed into heat energy. Bioluminescence, phosphorescence, and fluorescence are dependent upon the loss of the excitation energy in relatively large quanta, the quanta corresponding to the frequencies of vibration of light. Excited molecules lose their excitation energy more readily on collisions with molecules of electronegative than of electropositive gases. This accounts for the specific retarding effect of oxygen on the rate of certain photochemical reactions.

Only the absorption of light energy and not the mere presence of light

can cause photochemical reactions. The same holds true for heat energy. According to all experience, the absorbed energy is never remitted as isochromatic radiation in the reaction. In contradistinction to material catalysts which emerge unchanged at the end of the catalytic process, light and heat energy must be considered as reactants rather than as catalysts.

At equilibrium there are present the reactants and the products of the reaction unless some substance involved is separated as gas or solid into another phase. At equilibrium the rates of the reactions in the directions of synthesis and of analysis are equal. The presence of physical catalysts does not change the position of equilibrium but merely hastens the attainment of it. A source of energy such as heat or light may drive the reaction away from the equilibrium established without the reactant energy.

Independent of the thermodynamic character of the total reaction, the primary action of light is always essentially the same: the activation, generally, of one molecule or atom per absorbed light energy quantum. In endothermic reactions the number of molecules reacting is strictly limited by the amount of absorbed light energy; whereas in exothermic reactions the primary products formed in light absorption may cause a long sequence or chain of secondary chemical processes. The presence of activated molecules can be used to explain such chain reactions.

In photochemical reactions the rate of the chemical change is dependent upon the number of light quanta absorbed, that is, upon the number of activated molecules. The number of molecules reacting may be a small multiple of the number of light quanta absorbed, or in some spontaneous reactions the yields are large multiples of the absorbed quanta. In such chain reactions secondary processes occur which permit the transmission of the energy of activation to other molecules in the chain. The energy required for the molecular decomposition or the formation of intermediates in such cases does not come from the light quanta but from the energy liberated by the reaction. In this regard the light may be considered a catalyst in the chain of reactions. As many as 10^5 molecules can react per absorbed light quantum.

Although chain reactions are shown mainly in spontaneous exothermic reactions, there is no reason why the energy transfer from atom to atom or from molecule to molecule may not take place in endothermic reactions. The absorption of light energy by photosynthetic pigments may result in the storage of energy quanta which might be passed on to molecules of H_2CO_3 at a distance. Such a mechanism, if shown to occur in chloroplasts, might account for the storage of energy absorbed by chlorophyl molecules in the interior of the plastid and its transfer by

the mechanism used in chain reactions to the chloroplast surface or to the cytoplasm. This would obviate the static diffusion of CO_2 into the solid gel of the chloroplast and the diffusion out again of the oxygen and sugar.

In the active protoplasm many reactions proceed at the same time. The reactions may occur in series as in the decomposition of glucose to carbon dioxide, CO_2, and ethyl alcohol, C_2H_5OH, the oxidation of ethyl alcohol, C_2H_5OH, to acetaldehyde, CH_3CHO, the simultaneous oxidation of one molecule of acetaldehyde and the reduction of another molecule, $2CH_3CHO \rightarrow C_2H_5OH + CH_3COOH$. The energy liberated in one reaction may be used in another through the use of the mechanism of chain reactions. The energy liberated in the first exothermic reaction may not be degraded to heat at once so that it may be transferred by activated molecules to supply the energy for endothermic reactions.

Hydrolytic cleavages such as those catalyzed by enzymes involve but small energy transfers (Table 1), and they are generally easily reversible.

TABLE 1

THE THEORETICAL AMOUNT OF HEAT LIBERATED IN ENZYMATIC REACTIONS

Substrate	*End-products*	*Calories liberated*
Ethyl butyrate	Ethyl alcohol + butyric acid	1.2
Maltose	Glucose	3.8
Cane-sugar	Invert sugar	4.5
Salicin	Salicyl alcohol + glucose	5.3
Urea	Ammonia + CO_2	4.3
Hippuric acid	Glycocoll + benzoic acid	6.5
H_2O_2	$H_2O + O_2$	21.7

Reduction processes are mainly endothermic and vary greatly in the energy required.

Oxidation reactions are mainly exothermic; they may be spontaneous for this reason. The simultaneous oxidation and reduction reactions such as the Schardinger mechanism or the Cannizzaro reaction may involve about equal quantities of energy in the two phases. When energy is evolved or when the energy quanta involved in the two phases are equal, the reaction may proceed spontaneously, or it may be increased by catalytic substances. But when energy is required, light or heat energy must be available.

VI. *Light and Asymmetric Synthesis*

It is evident that light energy has been of the greatest importance in building up the organic world, since it is the principal source of energy.

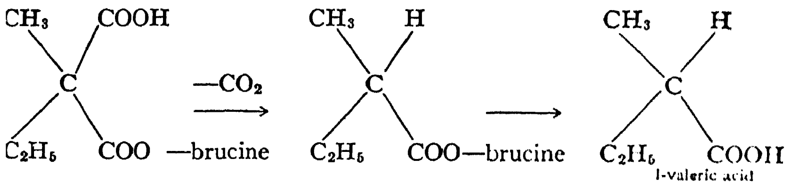
But light evidently determines the nature and configuration of the substance produced as well as the quantity synthesized. The substances in the organic world differ in their properties of absorbing light, and the energy so absorbed increases the instability of the absorbing substance. There are differences in the instability of optically active substances when exposed to light. Certain substances differ in their ability to absorb d- and l- circularly polarized light.

In organic chemical synthesis the substance formed is generally symmetrical in the configuration of the atomic groups of the molecule, or equal quantities of two d- and l- optical isomers are formed. When the environmental conditions are symmetrical in properties, the products are symmetrical arrangements. But in the synthesis by plants, the presence of asymmetrical compounds is quite remarkable. The sugars belong to the d- family of isomers, although certain members may show the opposite l-rotation owing to the nature of their formation. Evidently one family of configurations is formed to the exclusion of the other. This is evidence that some active agent in the environment is not symmetrical. When a catalyst of a reaction is asymmetrical, the product of the reaction will be asymmetrical also and will have a configuration corresponding to that of the catalyst. It is necessary to have, then, only the introduction of an asymmetrical catalyst to cause all of the optically active substances formed in its presence to be of the same configuration. Such an asymmetrical agent may be the light which has been found to be d- circularly polarized at the surface of the sea. This d- circularly polarized light tends to decompose one antipode rather than another and may have led to the synthesis of the first asymmetrical organic substances. Thus d- circularly polarized light is differentially absorbed by the d- and l- varieties of copper ammonium tartrate. The absorbed light energy then may increase the instability of one of these isomeric forms.

In the stereochemical configuration of substances produced in living cells when there is a possibility of stereoisomerism, the active form is generally present. The proteins are almost all l- compounds. The sugars all belong to the d- family. The tissues of living things are themselves asymmetrical, and in their action upon food materials select those whose stereochemical configurations best fit their own. Pasteur showed that certain fungi are unable to use l-tartaric acid as an energy source, whereas they actively oxidize d-tartaric acid. With the production of the first asymmetrical molecule the configuration of all substances formed in its presence would be determined, for asymmetry begets asymmetry of the same kind in the course of chemical reactions.

Asymmetric molecules in plants may be formed by the conversion of carbon dioxide and water attached to an asymmetric molecule such as

chlorophyl. By such a synthesis a carbohydrate chain may be produced, conforming to the configuration of the asymmetric agent. Such a method may be followed in organic synthesis in the laboratory. An active compound belonging to the desired configuration is joined to the radical which is to be used in synthesis, thereby forming a new asymmetric compound to which other radicals may be joined. When the original asymmetric group is split off from the molecule, the configuration of the newly synthesized part will have the configuration corresponding to that of the asymmetric agent used for the synthesis. Thus on heating the acid brucine salt of methyl-ethyl-malonic acid until no more carbon dioxide is liberated, it is found that the valeric acid which can be obtained from the residue contains an excess of the l- isomer. As shown in the formulæ below, the central carbon atom is not asymmetric, being attached to two carboxyl groups; but it becomes asymmetric in the second stage owing to the exchange of a hydrogen atom for one carboxyl radical with loss of CO_2. When the original active brucine radical is split off in the third stage, the optical activity of the new compound is due to asymmetric arrangement of groups around the central carbon atom, and this corresponds to the arrangement of the brucine.



That enzymes may be agents for asymmetric synthesis may be shown by the following syntheses. Emulsin acts upon a mixture of benzaldehyde and hydrocyanic acid, producing d-benzaldehyde cyanhydrin, and from this d-mandelic acid can be obtained. The original reagents were symmetrical, so the asymmetry of the product was caused by the catalyst, emulsin. Similar results are obtained when an active alkaloid is substituted for emulsin. The isomer produced depends upon the rotatory power of the alkaloid used. Thus when benzaldehyde and hydrocyanic acid are condensed in the presence of quinine (l-rotatory), l-mandelic acid is the product, whereas when quinidine (d-rotatory) is substituted for quinine, d-mandelic acid is formed.

VII. *Catalysis by Enzymes*

Of all types of catalysis, that effected by enzymes is most highly specific. Enzymes catalyze more complex reactions than do inorganic

catalysts. The catalyses of reactions by simple chemical substances tend to be general in their action, but the action of enzymes may be sharply confined to certain stereochemical configurations in the molecules undergoing change. This is probably for the reason that the enzymes are highly complex substances with specific arrangements in the molecule which are able to catalyze the reaction. A visual way of presenting this relationship between substrate and enzyme is to show a lock and key relationship, but no such actual mechanical arrangement has been proven. The relationship is more likely one of the arrangement of secondary valency forces in the two molecules. The enzyme and substrate form a labile compound, probably through secondary valence bonds. These unstable combinations are the active molecules in the reaction. The ability to form such combinations may be limited to certain stereochemical configurations. Part of the enzyme-substrate combination may remain undecomposed at the equilibrium point.

A difference of fundamental importance between enzymes and inorganic catalysts is the tendency of inorganic catalysts to drive the reaction in one direction whereas enzymes simply hasten the establishment of equilibrium between the reactants and the end-products. The equilibrium point established by enzymes is usually different from that established by inorganic catalysts such as H^+. Enzymes are thermolabile, while inorganic substances are usually thermostable. Enzymes are more sensitive to acid and alkali then inorganic catalysts. Furthermore, enzymes are much more active for unit weight than inorganic substances.

VIII. *Specificity of Enzyme Catalysis*

The information which we possess on the subject of enzymes is largely descriptive of types of action and the conditions affecting enzymatic processes. Two enzymes have been crystallized, urease and pepsin, but it is best to consider certain enzymes, such as oxidases, as types of action rather than as actual chemical identities. It is not necessary to assume the presence of a separate specific enzyme for each process. One enzyme may have more than one type of action, depending on the nature of the medium. While a few enzymes, such as invertase and catalase, have been shown to be highly specific, others attack large groups of related substances, for example the lipases or esterases which may hydrolyze almost any ester, the proteases which do not show specific action on proteins, and emulsin which may show three types of action. Absolute specificity seems more common in the enzymes which hydrolyze or decompose the carbohydrates than in other groups. But the more general action of the enzymes concerned with other groups may be due to mixtures of enzymes. It will be necessary to have crystalline preparations

and chemical identification before evidences on specificity will be of much value.

Evidences of the specific nature of enzymes is given by the different products formed from the same substrate by different enzyme preparations. The hydrolytic cleavage of raffinose by invertase yields melibiose and fructose, while hydrolysis with emulsin yields sucrose and galactose. Enzymes which catalyze the same process but which are derived from different plants may be affected differently by the physical conditions, such as temperature, or by the actual acidity, pH, of the medium (Fig. 30).

IX. *Classification of Enzymes*

Owing to their colloidal nature and to the formation of adsorption compounds, it is difficult to obtain pure preparations of enzymes. Pepsin and urease have been crystallized and may be definite chemical substances. But many types of enzyme action may be due not to specific substances but to certain colloidal conditions of substances. The peroxidases may be highly active colloidal forms of iron or manganese adsorbed on protein molecules. Enzyme preparations frequently show more than one type of action. The usual explanation of this is that, owing to difficulties of separation, a mixture of enzymes is obtained. Only few of the colloidal enzymes have been well isolated.

The classification of enzymes is based on their action on substrates. The action may be either in the direction of synthesis or of hydrolysis, since the function of the enzymatic catalyst is merely to hasten the establishment of equilibrium. The position of the equilibrium may be shifted by changes in the acidity of the medium, by temperature change, or by the mass action of one of the reacting substances.

The types of action of enzymes are various, but reactions involving the transfer of oxygen or of water, and molecular decomposition, are of greatest importance.

Preparations which showed enzymatic action were first named without any particular uniformity because sufficient information was not at hand to form a logical classification. The names pepsin and papain are reminiscent of this period. Then a classification on the basis of substrates was attempted, using the name of the substrate with suffix *-ase* to indicate the enzyme. When it became evident that synthesis as well as hydrolysis was effected by enzymes, the termination *-ese* was used to designate the synthetic type of action which led to the formation of a compound. It is obvious that there must be present in plants a mechanism for the synthetic as well as for the analytic reactions. In a few cases the conditions for synthesis have been studied. In many cases it is

necessary to assume that synthesis is a property of the protoplasm itself, since specific synthetic catalysts have not been demonstrated.

Table 2 gives a classification of the common enzymes, the substrates, with the conditions of their activity and the end-products.

X. *Relation of Enzymes to Temperature*

In enzymatic action it is generally found that the activity is confined to a relatively narrow temperature range. At temperatures as high as 70° C. the activity of most enzymes is destroyed in ten minutes. The destruction at temperatures below this is at a slower rate. Temperatures of 0° C. or below may allow little activity of the enzyme, but on returning to moderate temperatures the activity is generally found not to be affected. Freezing at the temperature of liquid air does not greatly affect the action of most enzymes. The range of the maintenance of enzyme activity is much the same as the temperature range for the existence of protoplasmic activity. The optimum temperature for the enzyme is taken as the temperature at which it shows the greatest activity, but there is concerned here also a time factor of enzyme inactivation which becomes prominent in its effect at high temperatures. Some organisms may be cooled to the lowest temperatures available and still survive, and some organisms live at temperatures near the boiling-point of water. Probably the ability of protoplasm to live at high temperatures is determined by the temperature maxima of its catalysts.

XI. *Relation of the Acidity pH to Enzyme Activity*

The enzymes show a limited range of acidity for their action (Table 3). This seems to be for the reason that enzymes are ampholytes and show differences in activity with differences in the acidity of the medium which determines their ionization. Some enzymes act when in the ionic condition; others act only in the un-ionized condition. The isoelectric points of the various enzymes differ greatly, so that changes in the acidity of the protoplasm may bring into action or stop the action of certain enzymes which it contains.

TABLE 2

CLASSIFICATION OF ENZYMES

(After Waksman)

Name of enzyme	*Optimum pH*	*Optimum temperature*	*Substrate*	*End-products formed*
I. Hydrolytic enzymes				
1. Esterases			Esters	Acids + alcohols
Lipases (bacterial)	7.2–9.0		Fats	Higher fatty acids + glycerol
Lecithinase	> 7		Lecithin	Cholin + glycerophosphoric acid
Cholesterinase			Cholesterin esters	Cholesterin
Chlorophyllase			Chlorophyl	Alcohol + phytol + chlorophyllid
Phosphatase (zymophosphatase)	6.5		Organic phosphorus compounds (fructose-diphosphate)	R.OH (fructose) + phosphoric acid
Glycerophosphatase	5.4–6.0	35° C.	Glycerophosphoric acid	Glycerin + phosphoric acid
Phytase	5.4–5.5	55° C.	Phytin	Inositol + phosphoric acid
Nucleotidase			Nucleotide	Nucleoside + phosphoric acid
Sulphatase			Ethereal sulphate	Phenol (or its homologues) + sulphuric acid
2. Carbohydrases				
(a) Polysaccharidases			Polysaccharides	Lower saccharides
Cellulase		20–70° C.	Celluloses	Cellobiose
Cytases	> 7		Hemicelluloses	Dextrins and monosaccharides
Diastase (amylase)	5.0–5.4	40–56° C.	Starch and dextrins	Dextrins and maltose
Inulase	3.8	55° C.	Inulin	Fructose
Pectinase (pectase)	4.3		Pectic substances	Reducing sugars
Seminase	> 7	35–40° C.	Mannogalactans	Mannose, galactose
Lichenase, etc.			Lichenin, etc.	Monosaccharides

TABLE 2—*Continued*

CLASSIFICATION OF ENZYMES (After Waksman)

Name of enzyme	*Optimum pH*	*Optimum temperature*	*Substrate*	*End-products formed*
(b) Trisaccharidases			Trisaccharides	Disaccharide + monosaccharide
Raffinase	4.5–5.0	30° C.	Raffinose	Melibiose + fructose
Gentianase			Gentianose	Gentiobiose + glucose
Melezitase			Melezitose	Turanose + glucose
(c) Disaccharidases			Dissaccharides	Monosaccharides
Invertase (α-fructosidase)	4.5	52° C.	Sucrose	Glucose + fructose
Maltase (α-glucosidase)	6.1–6.8	40° C.	Maltose	Glucose
Trehalase (α-glucosidase)	8.9		Trehalose	Glucose
Lactase (β-galactosidase)	4.2–4.6*	36° C.	Lactose	Glucose + galactose
Melibiase (β-galactosidase)	7.0†	50° C.	Melibiose	Glucose + galactose
Cellobiase (β-glucosidase)	6.0	46° C.	Cellobiose	Glucose
Gentiobiase (β-glucosidase)			Gentiobiose	Glucose
(d) Glucosidases			Glucosides	Glucose + other products
α-glucosidase (other than maltase and trehalase)			α-glucosides	Glucose + alcohol or phenol residue

TABLE 2—*Continued*

CLASSIFICATION OF ENZYMES

(After Waksman)

Name of enzyme	*Optimum pH*	*Optimum temperature*	*Substrate*	*End-products formed*
β-glucosidase (emulsin, phenol-β-glucosidase)	4.1–4.9	45–50° C.	β-glucosides	Glucose+alcohol or phenol residue
Amygdalase (oxynitrile-β-glucosidases)	6.0		Amygdalin (nitrile-glucosides)	Glucose+benzaldehyde+hydrocyanic acid
Myrosinase		45–50° C.	Sinigrin (myrosin)	Glucose+potassium-hydrogensasulphate+allyl isothiocyanatesa
Nucleosidase	7.5	37° C.	Nucleoside	Pentose+purine bases
Tannase	>7		Tannin (digallic acid)	Gallic acid
3. Enzymes acting upon proteins				
(a) Proteases				
Pepsin	1.8–2.2	38–40° C.	Proteins	Albumoses, peptones
Trypsin	7.0–9.0	60° C.	Proteins, albumoses, peptones, peptides	Peptides, amino acids
Erepsin	7.8	55° C.	Albumoses, peptones, peptides	Amino acids
Plant and microbial protease			Proteins, peptones, peptides	Peptones, peptides, amino acids
(b) Nuclease			Nucleic acids	Sugar, purine bases and phosphoric acid

TABLE 2—*Continued*

CLASSIFICATION OF ENZYMES (After Waksman)

Name of enzyme	*Optimum pH*	*Optimum tempe a-ture*	*Substrate*	*End-products formed*
(c) Amidases				
Desaminases			Amino acids	Hydroxy acids+ammonia
Desamidases			Acid amides	Acids+ammonia
Arginase	7.0	50° C.	Arginine	Urea+ornithin
Urease	7.2–7.9	55° C.	Urea	$(NH_4)_2CO_3$
Nitrilases			Oxynitriles	Aldehyde+HCN
II. Oxidases, oxydoreductases and zymases				
1. Oxidases				
Phenolase			Phenols	Quinones
Tyrosinase			Tyrosine	Melanins
2. Peroxidases				
Peroxidase			Peroxides	Reduction products of peroxides and active oxygen
3. Oxydoreductases				
Schardinger enzyme		65° C.	Methylene blue+aldehyde	Leuco compounds
Aldehydases		60° C.	Aldehydes	Acids and alcohols
4. Zymases				
Zymase	6.2–6.8	28–30° C.	Glucose, fructose, mannose, galactose	Alcohol+carbon dioxide
III. Catalase	7.5–8.0	10–40° C.	Hydrogen peroxide	Water+molecular oxygen

* Almond

† Yeast

TABLE 3

OPTIMAL REACTIONS FOR THE ACTIVITY OF VARIOUS ENZYMES

Enzyme	*Source*	*Optimal pH*
Amylase (diastase)	*Asp. niger*	3.5–5.5
	Asp. oryzæ	4.8
	Asp. oryzæ	3.0
	Cabbage, carrot, white turnip	6.0
	Fusarium, *Colletotrichum*	6.0
	Malt	4.4–4.5
	Malt	4.3 (at 25° C.)
	Malt	6.0 (at 60° C.)
	Phaseolus	4.0–6.0
	Potato juice	6.0–7.0
	Yellow turnip	4.0–7.0
Autolysis of yeast	Yeast	6.1
Bacterial enzymes	Hemolytic streptococci	7.0–7.9
Carboxylase	Yeast	5.3–6.2
Catalase	Vegetables	7.0–10.0
Cellobiase	Fungi	About 6.0
Desamidase	Yeast (grown on asparagin)	7.6–8.0
Dextrinase	*Asp. oryzæ*	4.0
Emulsin		4.4
Emulsin (β-phenol glucosidase)		4.0–5.3
Endoenzymes	Pneumococci	7.0–7.8
Erepsin	Yeast	
α-glucosidase	*Asp. oryzæ*	5.8–6.6
β-glucosidase	*Asp. oryzæ*	About 5.0
Inulase		3.8
Invertase	*Asp. niger*	2.5–3.5
	Potato juice	4.0–5.0
	Yeast	4.4–4.6 (52.1° C.)
	Yeast	4.2 (22.3° C.)
	Fresh yeast cells	4.2–5.2
Lactase	Yeast	About 7.0
Lipase	Castor-oil	5.0
Maltase	*Asp. oryzæ*	4.0
	Beer yeast	6.6
Oxidase	Vegetables	7.0–10.0
Pectase	Fruit	4.3
Pepsin	Yeast	4.0–4.5
Peroxidase	Vegetables	7.0–10.0
Phytase	Grain	5.4–5.5

Table 3—*Continued*

OPTIMAL REACTIONS FOR THE ACTIVITY OF VARIOUS ENZYMES

Enzyme	*Source*	*Optimal pH*
Protease	*Asp. oryzæ*	5.1
	Bacteria	6.0–7.0
	Malt	3.7–4.2
	Papain	5.0–7.0
Proteolytic enzymes	Yeast	6.7–8.5
Raffinase	Yeast	4.0–5.0
Trypsin	Yeast	7.0
	Yeast	8.0
Tyrosinase	Potato	6.5–8.0
Urease	Soy-bean	About 7.0
Zymase	Living yeast	4.5–6.5
Zymophosphatase	Yeast	6.2–6.6

The enzymes are highly colloidal catalysts of vital reactions which are characterized by thermal instability and sensitivity to those agents which act as protoplasm poisons. Of much importance in the killing of protoplasm at high temperature and by certain toxic agents is the destruction or lack of coördination of the life processes through the effects on its enzymatic system. Certain Phormidia which do not make use of enzymes for the catalysis of their reactions may live at temperatures near the boiling-point of water, and certainly 30–40° C. above the killing points of higher plants. Also these algæ live in the presence of free hydrogen sulphide, which is toxic to higher organisms.

XII. *Combinations of Enzyme and Substrate*

Enzymes act probably by forming adsorption compounds with the reactants, bringing them into closer contact so that chemical transformations can occur. The compound of invertase and its substrate, sucrose, is very labile and readily breaks down with the introduction of a molecule of water to form glucose and fructose. That a combination of enzyme and substrate is formed becomes evident in a latent period preliminary to the appearance of the products of the reaction.

That enzymes form adsorption compounds is easily demonstrated. In fact, selective adsorption may be used to separate enzyme mixtures. Invertase is quantitatively adsorbed by electropositive adsorbants, such as $Fe(OH)_3$ and $Al(OH)_3$, both in acid and alkaline media. Invertase evidently is ionized as an acid. Pepsin is also acid in nature. It may be adsorbed on infusorial earth, but it is not adsorbed by kaolin. Zymase is a neutral substance. It is not adsorbed by $Fe(OH)_3$. Lipase can

be adsorbed quantitatively on charcoal, in either acid or alkaline media.

Trypsin and malt amylase are amphoteric colloids. They are completely adsorbed in neutral or acid reactions by kaolin and infusorial earth, but are incompletely or not at all adsorbed in alkaline media.

Invertase is just as active whether it is dispersed in colloidal solution, or adsorbed on a colloid, saponin, or adsorbed on a solid, charcoal.

XIII. *The Autocatalytic System of Cells*

Living protoplasm may be considered as an autocatalytic substance; it transforms inorganic substances into living materials. The system to maintain itself must build up as fast as it is torn down, and to have growth of the protoplasm the constructive phase must continually be the more active. The mechanism of the formation of the first portion of this autocatalytic system has not been elucidated, but once given a mass of living protoplasm on the earth, further synthesis is the most common property of it. There is so gradual a change from non-living into living systems that it is not impossible to believe that if the proper conditions should be given, life forms would always be produced. Perhaps conditions on the earth at the time of the first formation of protoplasm were different from those of the present, for under present conditions no new protoplasm forms from entirely inorganic material. The synthesis of carbohydrates, amino acids, and their derivatives are all possible without the intervention of live protoplasm, and perhaps before living forms entered the earth there was a considerable accumulation of such organic materials, which might be united into autocatalytic protoplasm under conditions now unknown to us.

Certainly the most primitive organisms make use of simpler catalytic systems than the complex enzymes and photocatalysts of higher plants. The important place of sulphur, iron, and manganese as catalysts in oxidations and energy transformations is just now becoming recognized. The relation of the acidity of the medium has been recognized as of much importance for catalytic systems. The functions of definite organic substances as catalysts have now been indicated; and even two enzymes, urease and pepsin, have been crystallized. So we are now in position to gain more definite information on enzyme action.

Besides the use of iron, sulphur, manganese, and copper as catalysts, the probable use of the temperature of the environment to speed up reactions should not be overlooked, for this may be the key to the solution of the question as to how living protoplasm originated. There are certain Phormidia living in hot springs at 90° C. or above which evidently do not use the enzymatic scheme for the catalysis of their reactions. Perhaps

enzymes are unnecessary at such high temperatures, and inorganic catalysts, such as sulphur and iron, may serve as oxygen carriers. Othe vital reactions may be catalyzed by heat or light. These phormidia c not live when the temperature is much lower than 65°. enzymatic systems have not been established in them, if they are to be considered as most primitive, or they have lost the mechanisms for forming enzymes, if they are considered to have reverted from higher types of organisms. It seems most logical to assume that simple mechanisms should be most primitive, so we may have in these Phormidia examples of the first autocatalytic systems.

Physical agents as catalysts are generally not specific. A rise of temperature of 10° C. multiplies the rate of all physical processes about 1 to 1.41 times, and chemical reactions in general are speeded up 2–3 times by the same temperature increase. Hydrogen and hydroxyl ions show little or no specificity as catalysts; their effect is largely that of their mass action tending to increase the ionization, solubility, or aggregation of the reactants. Inorganic and physical catalysts may drive reactions in one direction owing to their mass action. The equilibria established by physical and inorganic catalysts may be different from those established by enzymes. Enzymes generally are highly specific; they may catalyze reactions of only certain stereochemical configurations. Enzymes generally may catalyze more complicated reactions than inorganic catalysts, owing to the highly selective type of action on certain reactions which are possible in the chemical system.

Enzymes are generally more sensitive to acid and alkali than inorganic catalysts. This is for the reason that many enzyme systems are amphoteric, and the ionization, solubility, and physical condition of the enzymes are much affected by acidity changes. Enzymes are generally much more active on the basis of unit weight than are inorganic catalysts. Invertase is about 5,000 times as active in the cleavage of sucrose as an equal weight of HCl. Enzymes may not lose their activity to any considerable extent in the course of the catalysis. Invertase has been shown to catalyze the hydrolysis of at least 1,000,000 times its weight of sucrose without decreasing its original activity to any appreciable degree.

In certain cases the biological catalysts seem to be highly active, loose compounds of inorganic catalysts. The peroxidases may be manganese or iron adsorbed on protein. In hemoglobin, iron is loosely bound and is evidently the active agent as an oxygen carrier. In chlorophyl, magnesium is the key substance. Both of these substances contain the inorganic catalyst in combination with pyrrol nuclei. Cytochrome contains groups similar to those of chlorophyl and hemoglobin.

Vital processes involve mainly reactions catalyzed by enzymes; simple

chemical reactions, such as neutralizations, are not of great prominence in metabolism.

XIV. *Distribution of Enzymes*

Since enzymes in many cases have been shown to be complex molecules or substances activated by adsorption on complex molecules, it is to be expected that conditions affecting the state of division of the colloidal particles will be of importance in determining the activity and distribution of enzymes in different parts of cells. Enzymes which are soluble in the external medium and for which the limiting surface of the protoplast is permeable, may diffuse out from cells. Cells are thus capable of effecting digestions outside of the protoplast. Such diffusible enzymes are called *exoenzymes*. They may catalyze reactions in the medium surrounding the cell. Other enzymes either are not soluble in the usual aqueous medium which surrounds the protoplast or consist of such large particles that they are not easily diffusible. These enzymes act mainly within the cell; they are *endoenzymes*. Endoenzymes, such as invertase, may be liberated upon autolysis of the cell. Cells may be killed without stopping the action of endoenzymes. Yeast cells killed with acetone may still retain their zymase activity. In some cases the endoenzymes may be extracted by grinding the cells in non-aqueous solvents. Lipase may be extracted with glycerin. Probably in this case lack of solubility in aqueous phases prevents diffusion from the cell. In many vital reactions no enzyme catalysts have been found, and it must be assumed that the reactions are effected by the protoplasm itself. This is the case of the more complex processes, which evidently require a highly specific course to be followed through the multitude of possible chemical transformations. The protoplasm evidently directs the course of the reaction and may change the nature of the process from time to time, possibly by forming or removing the proper enzymes for certain reactions. The cell is not always at the mercy of the environment, but may determine the course of metabolism in most cases.

In some tissues the enzymes are present in certain cells and their substrates are in other cells. Thus in bitter almonds the emulsin and amygdalin are present in different cells, and the decomposition of the amygdalin is accomplished only when the tissue is macerated or the enzyme and substrate are brought together by conditions which allow their diffusion between the cells. Hofmeister assumed that even within the cell, enzyme and substrate may be confined to different compartments or separate phases of the cell system. Perhaps a better explanation of the regulation of enzyme action by the protoplast is the explanation on the basis of the inactivation of the enzyme by an inhibitor.

Processes of storage, digestion, and transport of food materials in plants are effected by the production of enzymes. To produce the hydrolyses required in the transport of substances, the hydrolytic enzymes must be present in the tissue concerned. If the same enzyme is also the synthetic catalyst, then conditions at the point of storage must be different from those at the point of digestion. Thus in the transport of starch from leaves, amylase and maltase must be active. At the point of storage of starch the synthetic action must overbalance the hydrolytic phase. Yet the nature of the enzymatic action, whether it is synthetic or analytic, must change with time, for with the germination of seed or tuber, etc., the condensed storage product must be mobilized again for transport.

Diastase is generally more abundant in leaves which are rich in starch than in those which operate at high soluble sugar concentrations. It is more abundant in leaves which are exposed to light than in shaded leaves. Barley grown in sand containing phosphate has two or three times as much diastatic activity as barley grown in sand with insufficient phosphate. Diastase is especially abundant in the seeds, leaves, and fruits of plants belonging to the LEGUMINOSÆ. *Phaseolus* and *Pisum* are especially rich in diastase. The diastase concerned in translocation is especially abundant in bean and pea leaves and reproductive organs. This translocation diastase, an amylase, acts especially on freshly formed starch in leaves. The diastase concerned in secretion is especially formed during seed germination, at the expense of the protoplasm and cell nuclei. This secretion diastase, amylopectase, differs in properties from translocation diastase; it acts on starch grains and on the paste formed from starch.

The failure of certain leaves, such as onion, to form starch during photosynthesis may be due to lack of the amylases, dextrinases, or maltase necessary for starch formation. Such leaves accumulate soluble sugars instead of starch. During the growth period, the leaves of *Beta vulgaris* contain invertase, diastase, and maltase; the stems, invertase, diastase, inulase, and emulsin; the roots contain diastase, inulase, and emulsin, but no invertase. Evidently the absence of invertase allows sucrose to accumulate in the root.

The proteases are probably of universal distribution in cells, because they have both synthetic and hydrolytic functions and must be present in all protoplasmic structures. These proteolytic enzymes are especially abundant in germinating seeds which have a high protein content, such as lupines.

Resting seeds contain enzymes in very small amounts or in an inactivated condition. But as soon as water is absorbed and germination

begins, there is rapid formation or activation of the necessary diastatic, proteolytic, lipolytic, and other enzymes. These enzymes are especially formed by the embryo rather than in the endosperm. The scutellum of monocotyledonous seeds is an especially active organ in the secretion of diastase and other enzymes for the digestion of the reserves of the endosperm.

XV. *Formation of Enzymes*

The amount of enzyme produced by cells depends not so much upon the nature of the cells as upon the substrate in which growth takes place. A strain of *Penicillium* forms invertase only when the nutrient medium contains calcium lactate. When grown on starch, it produces abundant diastase; in milk it produces very actively the proteolytic enzymes. Most bacteria and fungi produce the enzymes necessary for the utilization of the substrate on which they grow. The differences in enzyme formation by different organisms is mainly quantitative rather than qualitative. Yet not all organisms are capable of forming all enzymes, and this is used to advantage in differentiating between strains of bacteria.

Rarely is a new enzyme formed owing to the presence in the medium of a specific substance or to specific conditions. The adaptation of organisms to certain media may not consist in the formation of a new enzyme, but may be merely a change in the constitution of the enzyme complex or the configuration of one of its components. It is not necessary to assume the existence of a separate specific enzyme for each process. The same enzyme may catalyze various reactions at different rates. There may be different types of catalysis rather than a multitude of specific catalysts. The critical thermal increments of vital processes indicate rather widespread action of a few catalysts, such as iron, and hydroxyl ion.

Definite chemical groups must be present for the synthesis of certain enzymes. This indicates that these structures may be built into the enzyme. Closely related substances may substitute certain substances which lead to the production of some enzymes. *Aspergillus* and *Penicillium* in Czapek's solution do not form tannase, but if sucrose, tannic, or gallic acid is present, tannase is formed. The greater the concentration of tannic acid is, the more tannase is produced. Leucine is essential for the formation of urease by *Bacillus proteus*, but it is not necessary for catalase formation. Catalase formation is stimulated by the presence of lactic acid, yet catalase is not present in certain lactic bacteria. The acidity of the medium influences urease production in *B. proteus*. The maximum urease formation takes place at pH 7.

Invertase formation in yeasts is influenced by both the temperature and the acidity. The optimum temperature for growth of a certain yeast was at 23.5° C.; the optimum temperature for invertase formation was at 27.5° C. The optimum pH for invertase formation was at pH 5.6, while pH 4.0–4.6 is the optimum for invertase activity. Hence it must be borne in mind that there may be different optima for invertase formation and for its activity.

The presence of starch in the medium increases the diastatic activity of various fungi, while sugar inhibits it. The degree of such inhibition depends upon the concentration of the sugar and the type of organism.

There is a great abundance of diastase in leaves early in the morning, but the diastatic activity decreases as the temperature rises. This may be due to temperature inactivation or destruction of the diastase of the leaf by light exposure. Light exposure, especially to ultra-violet decreases the diastatic activity of cereals.

Yeast which otherwise cannot invert sucrose can invert it when grown in media containing equal amounts of sucrose and glucose. Also, yeasts which do not produce galactase may split galactose if an equal amount of glucose is present in the nutrient medium. The nitrogen source greatly influences invertase synthesis and the diastatic activity in *Aspergillus oryzæ* and *Penicillium camemberti.* The presence of traces of zinc greatly accelerates the formation of diastase in cultures of *Aspergillus niger.* Boron, even in a few parts per billion, greatly accelerates the diastatic activity in leaves, and thus favors the translocation of carbohydrates.

The presence of free oxygen greatly increases the zymase activity of certain fungi, although zymase itself functions under anaërobic conditions.

XVI. *Proenzymes or Zymogens*

Enzymes are frequently secreted in inactive forms called *zymogens* or *proenzymes.* These zymogens are later transformed by activating substances into the active form of the enzyme. Thus peptases and tryptases are secreted as pepsinogen and trypsinogen. Both of these zymogens have been isolated. Esterases are often present in cells in the inactive condition.

XVII. *Activation of Zymogens*

Zymogens may be activated by acids, alkalies, or other chemical substances, or by specific complex organic agents called kinases. Kinases are the most highly specific of all activators. Enzyme activation may involve the formation of complex organic salts with heavy metals. The effect of heavy metals, such as lead and manganese, in activating oxidizing

enzymes may consist in the formation of adsorption or true chemical compounds of the heavy metal.

The zymogens may be regarded not as preliminary stages in the synthesis of a complex enzyme molecule, but should be considered more properly as a combination of the enzyme with an inactivating or paralyzing substance. Activation then may be merely the removal of the inhibiting or paralyzing substance and is not necessarily a synthetic process. The transformation of an inactive zymogen to an active enzyme is an irreversible reaction.

XVIII. *Coenzymes*

Enzyme activity may be increased by the presence of certain substances which are generally specific for each enzyme. Such substance are called *accelerators* or *coenzymes*. The addition of minute traces of manganese salts causes a great increase in the activity of laccase. Perhaps in this case there is formation of an adsorption compound of manganese, which is the active agent. It is practically impossible to exclude the presence of manganese from the laccase preparation, so all of the activity may be due to such adsorbed manganese. Calcium salts are coenzymes of pectase. Probably calcium is necessary for the precipitation reaction produced by pectase. Asparagin increases diastatic activity; the reason for this is not clear. Phosphates increase the activity of zymase, perhaps because they increase the formation of hexose phosphates, which are intermediates in the reaction.

XIX. *Antienzymes*

Substances which have a marked effect in inhibiting the action of enzymes may be called *antienzymes*. These substances probably combine with the enzyme or some part of it to render it inactive. Thus cyanides may inactivate the iron necessary for the activity of oxidizing enzyme systems. Adsorption reactions by various substances may cause them to act as antienzymes, by their being adsorbed upon the active enzyme surface to the exclusion of the substrate.

PART I

PART I

GENERAL METABOLISM

CHAPTER I

ABSORPTION AND SYNTHESIS

The vital reactions of the plant are carried out in a complex system of phases, some liquid, such as the cell vacuole, some gels, as the denser part of the protoplasm, and some solid phases, represented by numerous cell inclusions which may be in the crystalline state. It is possible to remove the cell sap as from a large celled Valonia and to feel that little change in composition has occurred during the removal. Similarly one can obtain crystalline proteins, starch grains, or other cell inclusions with apparently the same composition as existed in the cell. But when one proceeds to the analysis of protoplasm, the estimation of its composition in living matter becomes impossible with our present technique. The compounds of the protoplasm are often easily decomposed, or oxidized, or otherwise changed by the enzymes or other agencies already present in the cell. The methods of analysis themselves change the physical properties and chemical constitution of the protoplasmic substance. The living substance itself is continually undergoing change. Everything in the protoplasm is in a continual state of flux. There is change of chemical constitution and physical properties from one moment to the next, with even slight changes in the environment, or even in a perfectly constant environment. We can have a condition of complete cessation of change in the protoplasm only after it has ceased to be alive. It is not possible to find a living protoplasmic molecule, a search for which has often been attempted. The smallest living unit is the cell itself. Cytoplasm may live for a time without a nucleus, or the nucleus without cytoplasm, but such independent existence of either is not prolonged, and there is usually no growth of the separated part. The cell is the unit whose reactions we shall study.

Is it not possible that the substances making up the cell may be bound by secondary or primary chemical valencies so as to make a unit, when it is known that the units of crystals are so united? We have been inclined in the past to think of chemical union as being a much closer

association of constituents than the association of substances by adhesion or cohesion, but certainly chemical reactions may be influenced by the latter classes of phenomena. Possibly we should consider that the living unit of protoplasm is the mass of phases capable of being held together and coördinated by all of these forces. When a certain protoplast by its autocatalytic reactions has increased its size to a certain value, there is an increasing tendency for a release of the forces which tend to hold the mass together. This finally results in cell division, with a considerable regularity in the determining limit for size in any certain species of plant. The individual life units are neither as large as houses nor as small as single molecules. The size which may be attained by the unit is relatively limited. The disruptive tendencies finally overbalance the tendencies to remain connected, and the cell divides completely or establishes independent centers of activity within a supposedly common mass of cytoplasm.

The cells of a higher plant can all start from a single fertilized egg. The somatic cells change in size and structure according to the specific function which they perform in the division of labor of the organism. The size and structure is adapted to the function of each tissue. Perhaps the functions of the cells determine their differentiation in the tissues of the plant. When cells take on a new function, they may change their morphology accordingly. After differentiation has progressed to modify the cell greatly from the meristematic form, a change of function or morphology of such a cell may be impossible. Cells become stabilized with age into specific tissues from which they may not be able again to become meristematic or capable of reproducing the whole organism.

However simple or complex cells may be, they all show the fundamental physiological properties of living matter: automatic self maintenance, growth, and reproduction. To draw the line between the living and non-living things is indeed difficult, for they show many properties in common, but when man learns to put together non-living systems so that they show these fundamental physiological properties, we shall probably concede that he has created a living thing. But at present, living things come only from living things. There is no creation of living matter *de novo* which we have been able so far to detect in the earth, however much such origin in the past may be postulated by known cosmic conditions.

Living protoplasm catalyzes the formation of more living substances; in fact, it seems that this process has been followed from the very beginning of life on the earth, for all living things show genetic relationships with each other, whether plant or animal.

The reactions whereby new protoplasm is built up from non-living

materials with the absorption of energy are grouped under the name *anabolism*. The processes leading to the utilization of the stored energy with the physical and chemical mechanism for accomplishing it are differentiated by the term *catabolism*. Both the constructive and destructive phases may be described as *metabolism*, a term which is synonymous with vital processes.

Metabolism, at least as far as we have been able to discover, is carried on mainly by the interaction of chemical substances of the cell through catalysts, and especially by means of those thermolabile biological catalysts known as *enzymes*. The simple combination of chemical substances by neutralization, esterification, or similar processes, and the decomposition of these substances by reversal of the reactions, is too slow in many cases for maintaining the metabolic rate. The hydrolysis of an ester such as ethyl butyrate in water would take a very long time to reach the half-way point toward completion, but the introduction of a catalyst such as lipase enormously increases the rate of the reaction. The effect of the enzymes may be much greater than that of chemical catalysts such as the acidity of the medium. The enzyme lactase is 5,000 times as effective in hydrolyzing lactose as an equal weight of HCl.

The rate of chemical reactions in general is multiplied by 2 or 3 for each rise in temperature of 10° C., a rule which was formulated by Van't Hoff. The temperature coefficient of a chemical reaction (Q/10) is commonly known as the Van't Hoff coefficient. Accordingly, if a chemical reaction was proceeding at rate 1 at 0° C., at 10° C. it would proceed at a rate 2 or 3 times as fast. At the usual temperatures for growth, 20°–30° C., this same reaction would proceed at a rate of from 4 or 9 to 8 or 27. At a temperature of 90° C. this reaction would proceed at a rate lying between 2^9 or 512 and 3^9 or 19,683. In the hot springs of Yellowstone Park, organisms have been found living at 90° C. or above, and they seem to be devoid of the usual thermolabile enzymes found in ordinary plants. Possibly the use of enzymes to catalyze reactions at such high temperatures is not demanded because the chemical reactions required for metabolism are already proceeding at a high rate.

I. *Plant Metabolism in the Stages of Plant Evolution*

A characteristic of metabolism of the higher green plants and, in fact, also of many lower plants without chlorophyl is that the synthetic phases overbalance the phases which are destructive of organic compounds. The metabolism of such plants leads to accumulation of organic substances in nature. Green plants may be contrasted with animals, especially in this regard. The animal metabolism and the metabolism of many bacteria and fungi are entirely at the expense of the photosynthetic

activities of the green plant. Plants are said to be autotrophic when they synthesize their own organic compounds from the inorganic by means of the absorption of radiant energy, as in photosynthesis, or by the oxidation of reduced inorganic substances, as in chemosynthesis. Plants which ordinarily use only elaborated carbon compounds for obtaining energy are known as *heterotrophs*. The autotrophic type of metabolism must have preceded the development of heterotrophic nutrition in the evolution of plants.

The simplest organisms coming in upon a barren earth must have had a type of metabolism somewhat different from the complex forms which have now evolved. Such simple organisms must have been able to utilize the inorganic materials at hand under the conditions offered by the environment then existing. It cannot be expected that they appeared fully supplied with a system of enzymes, photosynthetic pigments, etc., such as we find in the modern higher plant. The first plants most probably had the ability to fix atmospheric nitrogen and to derive energy by the oxidation of reduced compounds already existing in nature, such as hydrogen sulphide or ferrous iron. Some of the simplest forms now extant, the blue green algæ, and certain bacteria related to this group, still make use of this type of metabolic reaction. Some of these plant forms have protoplasm which possesses properties not shown by the protoplasm of higher plants. Example is found in the ability of certain Phormidia to fix gaseous nitrogen, to live chemosynthetically, and to grow at temperatures sufficiently high to coagulate and kill the protoplasm of common higher plants, and also high enough to destroy the action of all of the enzymes.

In the course of evolution the primitive forms evidently have made use of new mechanisms for carrying on metabolism. A first great advance was probably the use of enzymes to catalyze reactions at comparatively low temperatures. A second important advance was in the use of photosynthetic pigments for the absorption and storage of the energy of light. Chemosynthesis probably preceded photosynthesis. The production of bacteriopurpurin, if this substance has photosynthetic action, was among the first trials, but this did not lead to as much success as the production of chlorophyl. We can definitely say that such organisms, capable of forming organic substances by photosynthesis or chemosynthesis, necessarily preceded both the animal forms and such heterotrophic plant organisms as the fungi. The assumption of a photosynthetic function by plants has led to the major developments of the organic world.

Not always has advance in the scale of evolution been accompanied by the gain of a new metabolic mechanism. All higher plants seem to have lost the ability to fix atmospheric nitrogen, and this was a very

serious loss when it is remembered that this gaseous form of nitrogen is the most abundant on the earth. The acquisition of nitrogen compounds is one of the major limiting factors in plant growth. It would seem that retention of this ability to fix atmospheric nitrogen must have militated against the performance of some other important function, such as photosynthesis. For if it did not, why should not some higher forms still possess this ability?

In like manner many higher plants have lost the ability to endure extreme fluctuations in temperature by adaptation, as in the process of hardening to increase their resistance to freezing temperatures.

As we proceed forward in the scale of plant evolution it becomes evident that there is a gradual increase in the complexity of the organic system. Entirely new compounds are found. The production of lignin by the pteridophytes marked a change from the evolutionary failures of the mosses to the dominance of the plants of the coal measures. Lignin allowed expansion of the plant to great size, enabling it to spread into the air large surfaces capable of photosynthesis. Algæ living in the sea had already reached such size without the use of lignin, on account of the greater buoyancy of the water. But large size in land plants did not occur until this important structural substance was developed. The greater complexity of compounds in the higher plants is evidenced also by the production of such compounds as the alkaloids which are mainly found in the dicotyledonous plants. There is promise that the whole path of the evolution and descent of plants may be found through the chemical relationships of the plant proteins as determined in serological reactions. As a result of his experiments on the relationships of plant proteins, Mez has proposed the scheme of plant relationships given in the diagram (Fig. 46).

That species and genera of plants differ in the fine points of their metabolism is shown by the multitudinous differences in the products derived from plants. The potato is valued largely for the starch which it produces; the artichoke stores inulin instead. Differences in the fine points of metabolism lead to the production of substances of widely different properties.

However much we may wish to do so, we cannot trace minutely the change of one type of metabolism into another. We may hope first to trace the transition of one genus of plants into another and to learn the causes underlying each transition. Evidently, the finer type of metabolism carried on is due to genetic inheritance, and this is bound up with the chromosomes of the nucleus. The nucleus has been considered as a governing body for cell activities. Since the production of certain substances in plants may depend upon the presence of particular parts of

chromosomes in the nucleus, it would seem that the mechanism controlling or inciting a particular reaction of metabolism, such as the production of anthocyanin, must be resident in the particular chromosome, perhaps at a particular position. But we have far to go to the understanding of such things.

II. *Development of the Ideas of Plant Metabolism*

The history of plant nutrition forms one of the most interesting phases of the development of scientific thought. The Greeks at the time of Aris-

FIG. [illegible] Bernard Palissy, [illegible]

"You will admit that when you [illegible] dung into the field, it is to retu[illegible] the soil [illegible]ething that has been taken away. . . . When a plant is burned, it is reduced t[illegible] salty [illegible] called alcaly [illegible] and philosophers. . . . Every sort of plant without exception [illegible] kind of salt. Have [illegible] seen certain laborers when sowing a field with wheat for the second yea[illegible], burn the unused straw which had been taken from the field? In the ashes will be found th[illegible] that the st[illegible] out of the soil; if this is put back, the soil is improved. Being burnt on t[illegible]d, it returns to the soil those substances that had been taken away."

Recepte véritable par laquelle tous les hommes de la France pourront apprendre à multiplier et augmenter leurs thrésors. 1563.

totle (384–322 B.C.) had observed that plants grow by materials which they take up from their environment. Since the nature of gases and of the air was not known, the Greeks naturally concluded that the soil

must furnish the nutritive elements to plants. Their ideas of plant nutrition were too much influenced by the observations which they could more easily make on animal nutrition. It was considered that the soil contained substances of the nature of humus which the plant absorbed and built into its structure. But since plants differed from animals in

FIG. 4.—Joannes Baptista van Helmont, 1577–1644.

"I took an earthen vessel in which I put 200 pounds of soil dried in an oven; then I moistened with rain water and pressed hard into it a shoot of willow weighing five pounds. After exactly five years the tree that had grown up weighed 169 pounds and about three ounces. But the vessel had never received anything but rain water or distilled water to moisten the soil when this was necessary, and it remained full of soil, which was still tightly packed, and, lest any dust from outside should get into the soil, it was covered with a sheet of iron coated with tin but perforated with many holes. I did not take the weight of the leaves that fell in the autumn. In the end I dried the soil once more and got the same 200 pounds that I started with, less about two ounces. Therefore the 169 pounds of wood, bark, and root arose from the water alone."

From the translation in E. Russell's "Soil Conditions and Plant Growth" of Van Helmont's, *Complexionum atque Mistionum Elementalium Figmentum* (in his *Opera Omnia*).

producing no excrement, it was necessary to add that the plant took from the soil by selective absorption only those substances which it used for making its structure. This humus theory of plant nutrition held

sway until the introduction of the experimental method in plant physiology nearly two thousand years later.

Palissy (Fig. 3), in 1563, made the observation that manures, and the ash of plants when applied to the field, increase the growth of crops.

FIG. 5.—John Woodward, 1665-1728. (By courtesy of the British Museum.)

"Vegetables are not formed of water, but of a certain peculiar terrestrial matter. It [illegible] be that there is a considerable quantity of this matter contained in rain, spring, and river water, that greatest part of the fluid mass that ascends up into plants does not settle the[illegible] but passes through th[illegible] pores and exhales up into the atmosphere: that a great part of the terre[illegible]rial matter, mixed with passes up into the plant along with it, an[illegible] that the [illegible]ant is more or less augmented in proportion as th[illegible] water contains a greater or less quantity of that matter; from all of whic[illegible] we may reasonably infer, tha[illegible] earth, and not water, is the matter that constitutes vegetables."

Thoughts and Experiments on Vegetation. Phil. Trans., XXI, 382-392 1699.

This observation was followed almost a century later (1650) by the search by Glauber for substances in manures which produced an increase in plant growth. Glauber succeeded in isolating saltpeter (potassium nitrate) from soil on which manure had been lying. On the application of this saltpeter to the soil he got enormously increased growth. The fact that the saltpeter came from the urine or feces of animals indicated that saltpeter came from the food of animals, that is from plant materials. This was the first definite connection of chemical substances with plant

nutrition. The brilliancy and exactness of Glauber's experimentation led to an accurate conclusion because he dealt with substances whose chemical technique was partly known by chemists of his time. Previously, the classical experiments of Van Helmont (1577–1644) (Fig. 4) had led to the erroneous conclusion that the dry weight of plants came mainly from water, for the reason that the nature of gases was unknown and the possibility of atmospheric gases supplying substance to the plant was

FIG. 6.—Marcello Malpighi, 1628–1694.

never suspected. Van Helmont's conclusion from his experiment was perfectly logical and his technique good, considering the status of chemical knowledge at his time.

An accurate experiment conducted upon the influence of water derived from various sources upon plant growth was conducted by John Woodward (Fig. 5) in 1699. He grew spearmint in rain-water, in water from the Thames River, in sewage water from the Hyde Park, London, conduit, and in a water culture made from the latter sewage water, with the addition of garden soil. The gain in weight in grams of spearmint plants

grown during 77 days in these solutions was as follows: rain water $17\frac{1}{2}$, Thames water 26, sewage water 130, sewage water plus soil 284. All of the cultures had an abundance of water, yet they showed marked differences in growth. Consequently he stated that Van Helmont's conclusion that the increase in plant substance came from water was erroneous. He concluded that it was the peculiar terrestrial matter contained in

FIG. 7. Stephen Hales, 1677–1761.

"And nature seems to make use of the like artifices in vegetables, where we find that air is freely [illegible] in; not only with the principal fund of nourishment at the root, but also thro' several parts of the [illegible] of the vegetable above ground; which air [illegible] nd in [illegible] elastick state [illegible] t freel[illegible] al vi[illegible]il through the larger *tracheae* of the vine; and is thence doubtless carried with the sap into [illegible] where being intimately united with the sulphureous, saline, and other particles it forms the [illegible]ative duct[illegible] matter, out of which all the parts of vegetables do grow." (P. 248.)

"We find by the chymical analysis of vegetables, that their substance is compounded of [illegible] salt, water, and earth; which principles are all endued with mutually attracting powers, and [illegible] of a [illegible] portion of air, which has a wonderful property of strongly attracting in a fixt state, or of repelling in an elastick state with a power which is superior to vast compressing forces; and it is by the [illegible]mbinations, action and reaction of these principles, that all the operations in animal and vegetable bodies are effected." (Pp. 319–320.)

Statical Essays. Vol. I. Vegetable Staticks. London, 1731–33.

the water which produced growth, and that growth was proportional to the amounts of this terrestrial matter which the water contained. Had he used the quantitative technique of Van Helmont in weighing the dry matter in the water as well as the plant, he might have drawn still more baffling conclusions. But among other things, Woodward made

the important observation of the water requirement for plant growth, and found that transpiration accounts for the loss of water from the plant. Malpighi (Fig. 6), in 1671, taught that food was taken up in a crude form by the roots, conducted by the fibrous elements of the wood, and was elaborated into plant substance in the leaves. Observation and experiment had led to a fairly accurate conception of the functions of plant parts in the nutritive processes, but there was still wanting information on those elusive substances the gases, on the recognition of whose nature and chemistry further advancement depended.

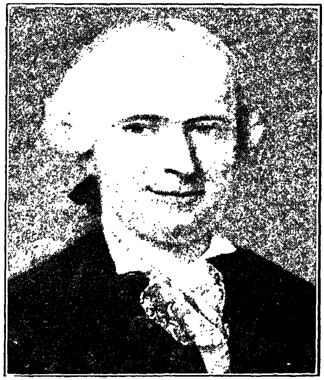


FIG. 8.—Jan Ingenhousz, 1730–1799. (After *Acta Horti Bergiani.*)

"... plants changing in the dark more respirable air into carbonic acid than they can digest, they throw out a large quantity of it, and thus render the air in contact with them less respirable, and that in the day they absorb with the atmospheric air so much matter of heat and light, or caloric furnished by the sun, that they cannot all digest it and therefore throw it out as superfluous, combined with the oxygen, which has thus acquired the nature of vital air."

An Essay on the Food of Plants and the Renovation of Soils. Agric. of the Counties of Britain, Hebrides, Central Highlands; Reports, etc. Vol. 14:1–20, 1794–1795.

Stephen Hales (Fig. 7), in 1731, in his "Vegetable Staticks," stated that air is "wrought into the composition" of plants. In 1771 Joseph Priestley, in experimenting upon the purification of the air by plants, stated "that plants instead of affecting the air in the same manner with animal respiration, reverse the effects of breathing, and tend to keep the atmosphere pure and wholesome when it is become noxious in consequence of animals either living, or breathing, or dying and putrefying in it." It was this same idea of purification of the air by plants which led to the experiments of the physician Ingenhousz (Fig. 8), in 1779, after the discovery of oxygen by Priestley. Ingenhousz discovered that light and green leaf pigments were necessary for the production of oxygen by plants. Priestley previously had demonstrated the production of oxygen by plants, but he had not observed the connection of light with the process. The final checking of the source of carbon for the plant we owe to Senebier who in 1782 showed that the increased weight of the plant in Van Helmont's experiment came from fixed air, carbon dioxide. From the work of Ingenhousz and Senebier it became apparent that plants under illumination produce oxygen, while in darkness they produce carbon dioxide. This is the first recognition of the essential differences between photosynthesis and respiration.

During this period Lavoisier had laid the foundations of modern

chemistry and had shown the true nature of water, carbon dioxide, nitric acid, and other substances containing the elements found in the atmosphere. We owe to Lavoisier the principle of the conservation of mass, that matter can neither be created nor destroyed. He had shown that animal respiration involved the production of carbon dioxide and water, and he had shown that respiration was essentially a combustion process, comparable to the combustion of organic substances *in vitro*. Nicolas T. de Saussure, by his excellent quantitative determinations of the gas exchanges by plants, in 1804 proved that not only was there fixation of carbon in the plant, but that hydrogen and oxygen, the elements derived from water, were fixed also. He also showed that besides water from the soil and carbon dioxide from the air, plants require various salts for their nutrition.

Boussingault, beginning in 1834, applied the quantitative chemical methods of De Saussure to rotations of field crops and kept an accurate check on the elements required for the growth of various crops. During this period also, Carl Sprengel conducted analyses upon the ash constituents of plants, to find which elements were essential to plant growth. The publication by Justus von Liebig (Fig. 9), in 1840, of his *Chemistry in its Application to Agriculture and Physiology* marks the beginning of a new era in the history of plant nutrition, not because Liebig himself contributed much to the subject, but on account of his forceful and authoritative manner of presentation. In place of the humus theory of nutrition we find that definite chemical constituents are spoken of by Liebig. These he compounded into a patented chemical fertilizer. The main contribution by Liebig to the ideas of plant nutrition was in his formulation of the Law of the Minimum. His statement of the Law of the Minimum is as follows:

FIG. 9.—Justus, Freiherr von Liebig, 1803–1873.

"The crops on a field diminish or increase in exact proportion to the diminution or increase of the mineral substance conveyed to it in manure.

"By the deficiency or absence of one necessary constituent, all the others

being present, the soil is rendered barren for all those crops to the life of which that one constituent is indispensable."

F. F. Blackman, in his expression of optimum and limiting conditions as affecting photosynthesis, extended this statement of the Law of the Minimum to cover all of the factors concerned in plant nutrition.

III. *Mineral Nutrients in Seeds*

The germinating seed uses the supply of mineral nutrients in the cotyledons or endosperm. Seedlings generally will produce a sufficient leaf area and root surface to establish the young plant without any mineral nutrients being supplied from the medium.

Usually the supply of carbohydrates is exhausted first when seedlings are grown in darkness in water culture. If the plants are grown in sufficient light, the nitrogen may be the first factor to limit growth. The deficiency of magnesium or potassium usually appears before deficiencies of iron, sulphur, or phosphorus.

IV. *Absorption and Use of Soil Substances*

After the exhaustion of the mineral nutrients of the seed, young plants are dependent upon the supply of mineral nutrients in their immediate environment. In the seedling stage the supply of nutrients in a particular small area will determine whether or not the plant can establish itself. Anyone can observe the growth of particular zones of vegetation over the different strata of sedimentary rocks. The composition of the stratum determines the survival of the species upon it. Of especial importance in this regard is the acidity of the soil, for some plants, such as alfalfa, *Medicago sativa*, require a neutral or slightly alkaline medium for their growth, while others, as rye, *Hordeum vulgare*, or sorrel, *Rumex acetosella*, may tolerate a considerable acidity. The acid-loving plants may be referred to as *calciphobes:* those which require a soil containing lime, *calciphiles*. How the acidity of the soil may affect plant distribution is shown in the following table (Table 4, page 50.).

In soil acidity there must be considered both the actual concentration of hydrogen ions (pH) and also the titrable acidity. The buffer action of soils varies greatly, so that different quantities of lime or other bases may be required to bring two soils to neutrality although they possessed at start exactly the same pH. The hydrogen-ion concentration determines the solubility and ionization of many important soil substances and the growth of plants (Fig. 10). Owing to differences in the composition of various soils, identical total acidity in two soils may produce great differences in the solubility of toxic substances in these soils and consequently cause differences in plant growth.

TABLE 4

AVERAGE FREQUENCY OF MEADOW SPECIES ON SOILS OF DIFFERENT pH VALUES
(From Olsen)

pH class		4 4.4	4.5 4.9	5 5.4	5.5 5.9			7 7.4	7.5 7.9	*No. of*
Deschampsia flexuosa.	86	68	40	..	..	..	..			
Calluna vulgaris.....	20	47	10	20	..	..	..			
Gallium hercynicum..	94	77	40	20	15	..	..			
Potentilla erectum....	67	90	63	73	48	43	10			
Agrostis canina......	..	100	100	73	93	100	..			
Festuca ovina........	..	100	47	35	20	20	30			
Anthoxanthum odoratum	33	47	70	80	83	70	27			
Deschampsia caespitosa	..	..	..	40	67	62	33			33
Circium oleraceum...	..	..	..	..	..	..	30		80	8
Angelica sylvestris...	..	..	..	..	..	55	33		30	
Tussilago farfara....	..	..	..	..	..	10	10		80	
Agrostis alba........	..	..	..	..	..	..	30			

If little leaching has occurred, soils which contain a good mixture of all kinds of rocks, such as glacial soils, will possess a balanced supply of

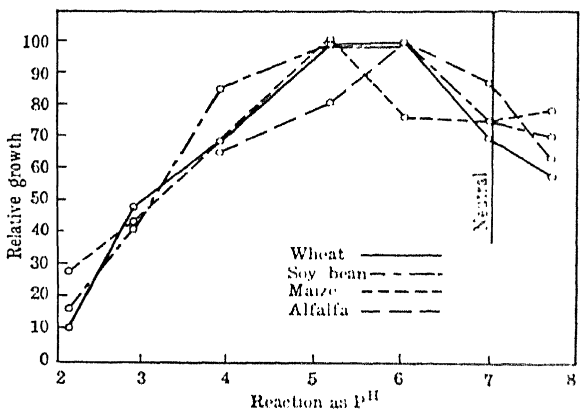


FIG. 10.—Relation between hydrogen ion concentration (pH value) and growth of crops in water cultures. (Salter and McIlvaine.)

minerals. In old soils, especially where the original rock was rather deficient in certain elements, these elements are liable to become de-

ficient. Because much of the rock is serpentine, the whole Piedmont region of the eastern seaboard of the United States is liable to show a calcium deficiency. There may be present in this soil also considerable free sulphur which, on oxidation, increases soil acidity. Because of the weathering of the rocks and the accumulation of nitrogenous compounds by the action of soil organisms, soils will, in general, be formed, provided the rainfall is neither deficient nor excessive for plant growth. Where the rainfall is excessive, many of the necessary constituents for plant growth will be leached out. Where the rainfall is deficient, the evaporation from the soil surface tends to accumulate soluble salts in the surface in excess, and these may be poisonous to plants.

When an analysis of the earth's crust is made, from as representative samples as we can hope to obtain, the following composition is shown (Table 5).

TABLE 5

AVERAGE COMPOSITION OF KNOWN TERRESTRIAL MATTER

	Lithosphere, 93 per cent	*Hydrosphere, 7 per cent*	*Average including atmosphere*
Oxygen	47.33	85.79	50.02
Silicon	27.74	....	25.80
Aluminium	7.85	....	7.30
Iron	4.50	....	4.18
Calcium	3.47	.05	3.22
Magnesium	2.24	.14	2.08
Sodium	2.46	1.14	2.36
Potassium	2.46	.04	2.28
Hydrogen	.22	10.67	.95
Titanium	.46	....	.43
Carbon	.19	.002	.18
Chlorine	.06	2.07	.20
Bromine	....	.008	....
Phosphorus	.12	....	.11
Sulphur	.12	.09	.11
Barium	.08	....	.08
Manganese	.08	....	.08
Strontium	.02	....	.02
Nitrogen	....	....	.03
Fluorine	.10	....	.10
All other elements	.50	....	.47
	100.00	100.00	

Of these elements C, H, O, N, P, S, Ca, Mg, K, Fe usually have been considered as essential for plant growth. Yet if the requirements of the whole plant kingdom are considered, many other elements must be present. It should be noticed that the second, third, and fourth elements in order of abundance are little used by plants.

V. *General Carbon Metabolism*

Carbon, which forms the basis for all living forms, represents only a fraction of a per cent of the earth's composition, and of this a major portion is bound in sedimentary rocks as carbonates. The carbon supply for organic synthesis is comparatively limited, and under natural conditions there must be a continual circulation of carbon as carbon dioxide into the plants, then to animals, or to bacteria and back again to carbon dioxide, which is the necessary form for photosynthesis by the green plant. From the action of sulphur and iron bacteria, there may have been a considerable accumulation of elaborated carbon and nitrogen compounds before the advent of chlorophyl bearing plants. This is especially indicated by the enormous accumulations of bog iron ore and of sulphur deposits. But in comparison with the green plants, this means of carbon assimilation is relatively unimportant. It has been suggested that the great luxuriance of vegetation during the carboniferous eras was due to greater concentrations of carbon dioxide in the atmosphere, accompanied by a higher temperature at the earth's surface. The raising or lowering of the earth's surface temperature should produce a greater change in the supply of carbon dioxide than of other elements important in plant nutrition. Higher temperatures on the earth should increase the carbon dioxide in the atmosphere by decreasing its solubility in water and by favoring the decomposition of carbonates. The concentration of carbon dioxide in the atmosphere is never very high, being only about three parts in 10,000 of air. An increase in the temperature of the carbonate rocks of the earth's surface should increase this concentration.

Most carbon compounds are rather easily oxidizable, so that plant and animal residues are quickly brought back into the cycle of carbon utilization. The waxes and resins from the surfaces of leaves and spores and the woody parts are the most lasting of the plant remains. It is these parts which are frequently found in coal deposits.

Of all the elements required for plant growth, carbon is the most likely to be the limiting factor. This is true, firstly, because its concentration in the atmosphere is so low that it is frequently a limiting factor in photosynthesis, and, secondly, because the presence of the

carbon-containing plant residues in the soil helps through the activity of soil micro-organisms to build up the supply of fixed nitrogen. The direct absorption of carbon compounds from the soil by green plants is of very minor importance in the carbon cycle. Carbon is available to autotropic land plants, principally as atmospheric carbon dioxide, and to submerged plants as carbonic acid and bicarbonates dissolved in the water.

VI. *Hydrogen and Oxygen in General Metabolism*

Although carbon is certainly the characteristic element of the organic world, still its abundance in living organisms is by no means as great as the elements of water: hydrogen and oxygen. The greater part of these elements found in living organisms occurs, however, simply as water. Water is required for the functioning of all active cells. The growing plant consists usually of more than half water, and this proportion may increase until the dry matter of the cell represents less than 1% of its green weight. Resting seeds and spores usually contain 10% or more of water, but germination depends upon the absorption of water. Water is a limiting factor of major importance for the growth of crops in many regions of the earth.

In addition to being a constituent of water, oxygen is required for the release of energy in the catabolic phases of metabolism. Without oxygen, life is impossible. It is the combination of carbon, hydrogen, nitrogen, sulphur, iron, etc., with oxygen which leads to the liberation of energy for organisms. For the normal respiration of fruits, the oxygen concentration in the air cannot be lowered beyond about one-third (8% of total) of the normal concentration present in the atmosphere (21% of total). Below a concentration of about 8% at ordinary temperatures, the supply of oxygen must be taken partly from oxygen containing compounds of the cell. This type of oxidation is known as intramolecular or anaërobic respiration. The anaërobic respiration of higher plants leads to the accumulation of abnormal oxidation products, and if continued for long will result in death of the tissue. The higher plants demand a high concentration of oxygen; they are obligate aërobes. The roots of plants may not receive a sufficient supply of oxygen for growth when the surface layer of the soil is not sufficiently permeable to oxygen. It is to be expected that a supply of oxygen may be made available to roots and stems from transport of the oxygen produced during photosynthesis. Many water plants have elaborate systems of air-chambers which apparently function as passages and reservoirs for oxygen. Owing to the numerous soil organisms, the oxygen of fertile soils is usually so low that some seeds will not germinate when they lie more than eight inches below the surface.

Certain fungi and bacteria have the ability to live either under aërobic or under anaërobic conditions. They may be said to be facultative aërobes or facultative anaërobes. Sometimes the mycelium may grow under anaërobic conditions but will not form fruiting bodies until free oxygen is available. Certain bacteria and fungi require the absence of uncombined oxygen for their growth. These forms are said to be obligate anaerobes.

Other than in compounds formed from the hydrogen which is contained in water, hydrogen is of very minor importance in plant nutrition. There are certain chemosynthetic bacteria, such as *Hydrogenomonas*, which can use the oxidation of elemental hydrogen as a source of energy. Elemental hydrogen is produced only under anaërobic conditions, generally in masses of organic matter isolated from the atmosphere. It is a product of butyric fermentation, and may be found in putrefying masses from which the oxygen supply is cut off, such as in canned foods or in bogs. Gaseous hydrogen is, however, so light that it is contained in the lower part of the atmosphere only in slight amounts.

VII. *General Nitrogen Metabolism*

Of nitrogen, by far the greater part exists in the gaseous state. Nitrogen makes up 79% of the atmosphere, but gaseous nitrogen is not available to higher plants directly. The ability to fix atmospheric nitrogen is found only in a relatively small number of the lower orders of plants. Of the organisms which carry on nitrogen fixation, the bacteria are of most importance, although certain fungi and blue green algæ are able to use the nitrogen of the atmosphere for the formation of their protoplasm. Only relatively small supplies of fixed nitrogen occur in the soil other than that which is found in the protoplasm of living organisms. A fertile soil contains such a host of bacteria, fungi, amœbæ, flagellates, and other organisms, that most of the nitrogen compounds of dead plants or animals have little chance to accumulate as nitrates or ammonia.

Nitrates usually represent about .006% of garden soils; in pasture soils the range is from .0002% to .002%, while in woodland soils the content is still less. Ammonia represents about .0001% of arable soils. This may be increased tenfold by applications of manure. The organic nitrogen compounds of the soil, chiefly the proteins of living organisms, represent about 0.15% in arable soils, 0.3% in pasture, and much greater percentages in bog and chalky soils.

Nitrates are the best supply of nitrogen for most plants, and in tilled soils it may be practically the only form of any considerable importance. Nitrates are all soluble, and we are concerned with the effects of the nitrate ion upon the plant. Nitrites might exist in soils, but only under very

abnormal conditions. They are found in sewage waters, but in oxidizing conditions suitable for the growth of higher plants they are absent. Nitrites may be absorbed and assimilated if the concentration is kept very low, but owing to the ease of their oxidation there may be a question as to their existence in the plant as nitrite.

High nitrate content of the soil leads to a rapid vegetative growth of thin-walled cells. The leaves are broad, dark green, and succulent. The plants are taller than normal, and the supporting tissues of the stem are reduced in proportion to the normal. The plants are especially non-resistant to fungus attack, possibly on account of the thin cell walls. Nitrate retards the production of reproductive parts, and leads to slow ripening. The yield of tubers, etc., is decreased, probably because the carbohydrate is so extensively used for cellulose synthesis. Nitrates favor an extensive root development. But although the mineral and carbon assimilating powers of the plant are increased, the yield of seed, tubers, or roots may be much decreased. If a sufficiently long season is given, increased yields of some crops will be produced. The first frost, however, usually cuts off the succulent plants grown in soils with high nitrate content.

The flowers and young fruits of tomato may be cut off by an abscission layer when nitrate is in excess. In general, the yield of leafy crops is increased by high nitrate applications.

VIII. *Carbon/Nitrogen Ratio*

It is evidently necessary to have a proper balance between the nitrogen supply and the carbon assimilation of plants to get normal development. If nitrate is in excess, the plants tend to be vegetative. A large part of the carbohydrate goes to form cellulose and protein constituents. If nitrate is lacking or deficient, there is accumulation of large amounts of starch. Either condition leads to unfruitfulness. A proper balance between nitrogen and carbon in the plant, or a proper C/N ratio, leads to normal development and a setting of fruits.

We shall have occasion to refer at greater length to nitrogen metabolism as well as to the special metabolism of carbon.

IX. *Absorption of Ash Constituents*

With regard to the chemical elements which are found in the ash of plants, we find a relatively small number in abundance, but traces of a great many chemical elements are found in plants. It is difficult to say which elements are essential for growth, for in many of the experiments made in the past the exclusion of traces of the elements has not been carefully carried out. Of the heavy metals, not more than spectroscopic

traces are tolerated by most plants. Still, some bacteria can grow in solutions of silver salts. In general, ten chemical elements have been considered essential for plant growth. These are C, H, O, N, P, S, K, Ca, Mg, Fe. But for certain plants at least, and possibly for all plants, a considerable stimulation of growth and other metabolic processes occurs if there are present, in addition to these ten, traces of B, Mn, Al, Si, Cu, As, Zn, I, Cl. In fact, a number of elements which are poisonous at relatively low concentrations, for example, copper, arsenic, or zinc, stimulate growth when they are present in a few parts per billion.

The total ash content of plants represents about 5% of the dry substance, but there is wide latitude in the ash content. Some plants, as spinach, pumpkin, etc., take up large amounts of salts from the soil when such are available. They are gross feeders. But other plants grown in the same soil may absorb much smaller amounts. Plants vary in their ability to absorb salts from the soil. In most fertile soils the salt content is relatively low. The soil solution has much the same composition as the river waters at times when there is little surface wash from rains. It may be expected that water which percolates through the soil will approximate the concentration of substances in the soil water. If the total concentration of the soil water corresponds to only .0001N KCl, the plant still can absorb most of the salts from this dilute solution. Maize can reduce the conductivity of such water by absorbing salts until the total concentration represents about .00001N KCl. About half of this residual concentration of ion-forming substances can be removed by boiling, and probably is carbonic acid. So that maize grown in a limited amount of solution makes an effective clean up of the ionized salts in the soil solution. Seedlings can maintain this extremely low concentration for a few weeks, but upon death, ions are given up to the solution again. Probably the vacuolar concentration of total ions is much above the ionic concentration in the external medium. The ability to hold the ions is lost by the protoplast after death. Different species of plants can absorb ions to different levels of concentration. The squash cannot absorb soil salts to as low concentrations as maize. It may be expected that plants will show differences in their ability to live in and absorb from soils which are deficient in nutrients.

X. *Soil Solution*

The salts present in the soil come from the solution of the constituents of the soil under the action of weathering and of soil organisms. An idea of the composition of the soil solution can be gained from the following table (Table 6).

TABLE 6

Nature of soil	*Moisture in soil*	*Parts per million of soil solution* K	PO_4	Ca	N
Fine sand..........	29.74	24.1	5.2	30.6	3.1
Loam..............	37.80	71.1	12.2	68.2	3.2
Clay...............	24.50	44.8	4.6	42.9	6.1
Peat...............	132.90	50.1	2.5	183.8	17.1

The solubility of the constituents of granites and gneisses is increased by additions of sulphur to the soil, which on oxidation produces sulphate ion. The solubility of limestones, dolomites, and other carbonate rocks is much increased by carbon dioxide excreted during the respiration of roots, and carbon dioxide also is produced by bacteria and fungi living either on roots in symbiosis as mycorrhizæ, or free in the soil. Probably other organic acids may be produced from mycorrhizæ by partial oxidation of cell-wall materials and thus aid in the solution of substances from the soil. Other bases may be dissolved in this manner also, for it may be expected that their solubility will be increased with an increase of the actual acidity (pH) of the soil solution, however small it may be.

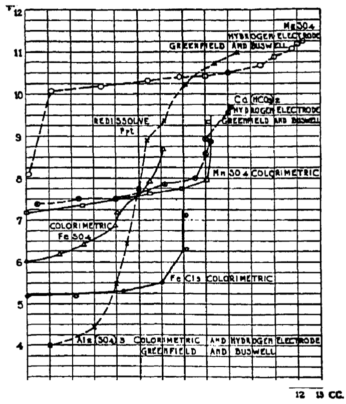


FIG. 11.—Precipitation of aluminium, ferric and ferrous iron, manganese and magnesium as hydroxides and calcium bicarbonate as calcium carbonate. (After Carr.)

That the soil acidity does affect the solubility of soil constituents is demonstrated by the solution and absorption of aluminium and iron in the acid spots of fields where the soil contains much clay. The absorption of excessive amounts of iron and aluminium and the toxic action of these substances in the plants can be decreased by lowering the soil acidity by additions of limestone or by the addition of substances, such as phosphates, which form insoluble compounds with Fe or Al. Aluminium will be present in soil water in about the following proportions at various acidities:

pH	4.87	5.14	5.30	5.50	6.90	9.01
Al_2O_3 ...	1.2	2.0	0.7	0.3	0.7	31.0

Iron is little soluble between acidities of pH 5.5 to 9.0, and will not produce toxic effects on plants if the soil reaction lies between these limits (Fig. 11).

XI. *Abundance of Soil Constituents*

Considering that all of the nutrients in the soil are available to plants, which, of course, is impossible, Hopkins has calculated the limiting time during which maize could be removed as a crop without replenishing the supply. In the average plow sole depth, $6^{2}/_{3}$ inches, over an area of one acre, or corresponding to 2,000,000 pounds of soil, the essential nutrients are present as follows (Table 7).

	Wt. lbs.	*Lbs. per 100 bu. maize*	*Year's supply*
Phosphorus.......	2,200	17	130
Potassium........	40,200	19	2,000
Magnesium.......	48,000	7	7,000
Calcium..........	68,800	1.25	55,000
Iron.............	88 800	0.30	300,000
Sulphur..........	2,200	0.22	10,000

Of these elements those in least abundance in relation to the requirements of the maize are phosphorus and potassium. In most soils these are the elements which are most likely to be deficient for all crops. Sulphur is no more abundant than phosphorus, but the demands for producing the crop are less. Furthermore, there are gaseous compounds of sulphur which lead to a thorough distribution of this element, so that sulphur deficiency is seldom found. The hydrogen sulphide produced by the decomposition of vegetable and animal remains under anaerobic conditions particularly tends to distribute the sulphur. Large quantities of sulphur dioxide are produced from the burning of coal and from the heating of iron pyrites. Not all of the beneficial effects of the application of sulphur to soils comes from the satisfaction of a sulphur deficiency. Part of the increased yield of the crop may be due to the solution of other soil constituents, such as the solution of potassium from orthoclase.

XII. *Adsorption by the Soil and Leaching*

The loss of certain ions through leaching from the soil in rain-water is reduced by the power of certain soil constituents to hold ions by adsorption. Potassium ion, phosphate ion, and ammonium ion are relatively strongly adsorbed by most soils. Sands have low adsorptive

power, clays adsorb K^+ and $PO_4^{\equiv}$ strongly. Colloidal double silicates of calcium and aluminium are probably responsible for the adsorption. Since the salts of potassium and ammonium are highly soluble in water, adsorption of these ions is of great importance in maintaining them in soils. Probably the cations K and NH_4 may replace Ca from clays in addition to their being strongly adsorbed on the surface of colloidal soil particles. Phosphates in neutral solution form relatively insoluble compounds with calcium and also with aluminium.

Soils in general have little power of holding by adsorption Ca^{++}, NO_3^-, Mg^{++}, Cl^-, $CO_3^=$, and SO_4^-. These are rather easily leached by rain-water. The rain-water is usually charged with CO_2, and this leads to the solution of the normal carbonates of calcium and magnesium by the formation of bicarbonates, which are much more soluble. These ions which are not held by soils make up the salt content of the efflux from the soil.

There is usually present but little potassium or phosphate in the soil efflux. The soil water from various types of soils contains usually from six to ten parts per million of phosphorus and about twenty-five parts per million of potassium. Long continued washing of clay soil with distilled water produces little change in this concentration.

The presence of sodium and magnesium salts increases the concentration of potassium in the soil efflux, probably by replacing it in adsorbing surfaces and also by substituting it in the double silicates of the clay.

XIII. *Differential Absorption*

If we analyze the ash of different genera or species of plants which grow in the same habitat, we find that the constituent elements are present in different proportions for each species. The elements absorbed are not present in the ratios in which they exist in the medium, even if water culture is used to ensure the same ionic concentration for each plant. Evidently there is selective absorption of the salts existing in solution. If we use in water culture a single pair of ions such as ammonium and sulphate, it becomes apparent that the two ions enter at different rates. When ammonium sulphate is applied as fertilizer to the soil, ammonium enters the plant to a greater extent than the sulphate ion. After a few years the soil becomes distinctly acid from the accumulation of the unabsorbed sulphate ion. Such production of soil acidity is due to the selective ion absorption as distinct from the differential adsorption of ions by soil colloids, and this physiological cause is just as important a factor as the other causes of soil acidity which have been much discussed recently by soil chemists. With applications of potassium

nitrate in neutral solution, the nitrate ion is absorbed more rapidly than the potassium ion, and the solution becomes slightly alkaline.

The roots of *Cucurbita pepo* in water culture showed the following absorption of various ions (Table 8).

TABLE 8

Salt	*Conc. gm. moles. per L.*	*Duration in days*	*Absorption in milligrams* Cation	Anion
KCl	0.03	6	23.38	40.68
$CaCl_2$	0.02	14	0.00	51.30
K_2SO_4	0.0188	6	11.6	18.07
$CaSO_4$	0.0165	10	0.00	1.08
KH_2PO_4	0.02	10	1.15	40.04
	0.032	12	1.10	78.03

This table shows especially low absorption of calcium and of sulphate ions. For certain ions, $NH_4^+S^=$ it has been shown that the acidity of the external medium is of great importance for the absorption.

XIV. *Donnan Equilibrium*

Assuming that mineral nutrients enter the plant in the ionic condition, an explanation of the differential absorption of ions by plants may be found in the conditions formulated by Donnan for the establishment of equilibrium across semipermeable membranes. The cells of the plant contain ions which may not pass through the cell membrane. Probably the protein ions are of major importance in this regard. When a semipermeable membrane separates two phases of a system in one of which there is an ion which cannot diffuse through the membrane, an equilibrium will be established when the products of the concentrations of each pair of oppositely charged diffusible ions on each side are equal. If the concentration of a non-diffusible anion is taken as R^-, and the concentrations of diffusible cations on each side of the membrane are taken as C_1 and C_2 and of the diffusible anions as A_1 and A_2, then equilibrium will be established when $C_1A_1 = C_2A_2$. Since on the side of the non-diffusible anion the concentration of cation is the sum of that held by the non-diffusible anion plus that held by the diffusible anion, while on the other side the concentration of the diffusible cations is only that held by the diffusible anion, the equilibrium condition can be fulfilled only if $C_1 > C_2$ and $A_1 < A_2$. Diffusible ions will pass through the membrane to establish this condition. There is an inequality of the concentration of the diffusible cations and anions on the two sides of the membrane. Under the Donnan equilibrium conditions there is a difference of potential set up across the

membrane owing to the differences in ionic concentrations as illustrated in the diagram.

C_1^+	‖	C_2^+	1 is + to 2^-
R^-	‖		
A_1^-	‖	A_2^-	
	membrane		$C \times A \quad C \times A$

The fluctuations of acidity of the protoplast should change the equilibrium point because it should change the concentration of protein ions in the cell.

At the equilibrium concentration shown by maize in water culture, it would appear that practically all of the cations and anions in the external solution pass through the plant cell membrane. The diffusible anion passing out from the roots to establish the equilibrium concentration is $CO^{3=}$. This would indicate that possibly the proteins of maize are present in the cell mainly as the anion, enabling them to hold the cations in equilibrium against the external solution. The anions in the external solution evidently are replaceable by $CO^{3=}$. Upon the death of the protoplast either the semipermeability is lost, or the concentration of the non-diffusible ions falls off, with resultant leach of ions from the tissue.

It is evident from the conditions produced by the absorption of seedlings in water culture, that the plant will absorb practically all of the ions present in the solution, independent of the nature of cation and of anion, provided the quantities offered are not in excess of the ability of the plant to absorb and utilize them. The conditions in the soil generally are such that the equilibrium point is never reached. The plant must have a supply of certain ions or it will be unable to maintain itself and will die after a few weeks. But different plants use greatly different quantities of each ion in the elaboration of the compounds peculiar to them. Some elaborate more organic sulphur compounds than others, some more chlorophyl, demanding magnesium, and so forth. Owing to change in the internal phases of the plant, due to increased size and the formation of new materials, there probably also is absorption from an excess of nutrients in the soil and under conditions in which equilibrium can never be established.

XV. *Selective Absorption of Certain Ions*

In a survey of the characteristic elements of plant ashes it is found that particular elements are especially abundant in members of certain

groups of plants which sometimes show genetic relationship. For instance, the grasses show the ability to absorb relatively large quantities of silicon. They may be called silicon plants. It seems that those plants which commonly produce high concentrations of soluble carbohydrates also show a high absorption of potassium. They may be referred to as potassium plants. We have seen already that certain plants have relatively high demands for calcium. The composition of the ash of these different types may be illustrated by the following table (Table 9).

TABLE 9

Per cent in ash of		*Salts of K + Na*	*Salts of Ca + Mg*	*Silicic acid*
Silicon plants...	Oat straw + grain......	34.00	4.00	62.08
	Rye straw..........	18.65	16.52	63.80
Calcium plants..	Tobacco (Havana).....	24.34	67.44	8.30
	Stems and leaves of pea.	27.82	63.74	7.81
Potassium plants	Sugar cane...........	88.80	12.00	....
	Artichoke..........	84.30	15.70	...

An indication that an equilibrium between plant and soil is never reached under natural conditions in the growth of plants is given by the change in proportions of constituents and of total ash content in the same organ of the plant at different ages. The following table gives the analysis of beech leaves at different times of the year (Table 10).

TABLE 10

Date	*Total ash per cent of dry wt.*	*Amounts of various elements in ash calculated as oxides per cent total ash*					
		K_2O	*CaO*	*MgO*	*Fe_2O_3*	*P_2O_5*	*SiO_2*
May 16..	4.1	42.1	13.8	4.3	0.8	32.4	
July 18...	4.7	17.1	42.3	5.6	1.4	8.2	
Oct. 15...	7.1	7.1	50.6	4.1	1.3	5.1	30.5

The increase of calcium and silicon with the age of the leaves is quite marked, as is also the decrease in proportions of potassium and phosphorus in the total ash. If the same number of beech leaves are taken at different periods, it is found that the potassium and phosphorus content per leaf is the same. In other words, there is practically no removal of these elements from the assimilating leaf. However, at the end of the growing season there is translocation of both K and P back into the stem. Between May 16 and July 18, the total proportion of ash to dry weight

did not increase, but in the later analysis the concentration is increased. When dead leaves are analyzed, it is found that a considerable part of the ash has either been withdrawn into the plant through the petiole or leached from the dead cells.

XVI. *Toxicity of Ions*

When plants are grown in water cultures in solutions of single salts, toxic symptoms become evident if the concentration exceeds certain limits. The toxic limit is exceedingly various. For salts of the heavy metals the toxic concentration is represented by a very few parts per billion of water. Algæ may be killed in ponds and water supplies by the introduction of exceedingly low concentrations of copper sulphate. For salts of iron, the toxic limit is a few parts per million, depending probably upon the actual concentration of the ferrous and ferric ions. For sodium, potassium, and magnesium salts the toxic concentration appears at a concentration between one thousandth and one ten thousandth normal. The salts of calcium do not become toxic until much more concentrated solutions are reached. Evidently the effect of the anion in combination with calcium is of major importance in the toxicity of its salts. In the other ionic pairs it is evident that the cation concentration is of major importance, provided that the choice of anion is limited to those usually of importance in soils.

XVII. *Antagonism of Ions*

The toxicity of the single cations is greatly reduced by admixture with other cations. The addition of calcium or aluminium to the solution decreases the toxicity of copper. The addition of calcium to solutions of potassium, sodium, or magnesium decreases the toxicity of these elements. This action is known as antagonism of ions. An example of the antagonism between aluminium ion and copper ion is shown by the following experiment: the hypocotyls of pumpkin (*Cucurbita pepo*) had a life duration of one hour in .005625n $CuSO_4$ solution; on the addition of .025n $AlCl_3$ the life duration was twenty-two hours.

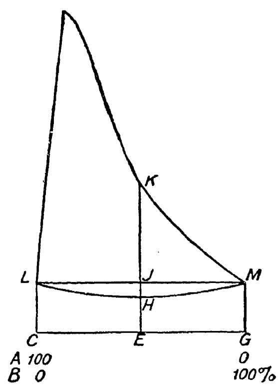


FIG. 12.—Curves showing the growth of roots in mixtures of equally toxic solutions of two salts, *A* and *B*. (After Osterhout.)

On mixing two equally toxic salt solutions in different proportions we may find that the toxicity is unaltered. As the proportion of the toxic concentration of one ion *A* in the diagram (Fig. 12) is decreased from 100

to o and the equally toxic concentration of another ion *B* is increased, if there is no change in toxicity the effects of the two ions are additive. The effect of one ion increases in exact proportion as the effect of the other decreases. This condition may be represented by the graph *LM*, the reciprocal of the toxicity being represented on the vertical axis. If, however, the toxicity is increased, the conditions may be represented by the graph *LHM*. If the toxicity is diminished, the condition may be represented by the curve *LKM*. This shows antagonism of the two ions, one tending to counteract the toxicity of the other. In making such mixtures it is found that there is a definite point of maximum antagonism between the components of the mixture. Growth will be better in the optimum mixture than at any other proportion between the two toxic constituents. For the antagonism of $CaCl_2$ and NaCl the optimum lies at the proportion Ca : Na : : 4.76 : 95.24.

XVIII. *Balanced Solutions*

Antagonism exists in mixtures of several ions. It is possible, therefore, to have rather high concentrations of salts in antagonistic mixtures without toxicity making its appearance. Particularly is calcium of importance in preventing the toxicity of high concentrations of Na, K, and Mg. If the calcium is depleted from soils high in these ions, the toxic effects appear. The soil solution becomes unbalanced in its constituent ions. A balanced solution may be established by the addition of ions antagonistic to those which are in excess. In additions of sodium nitrate to soils, it is advisable to add also calcium salts to the fertilizer to prevent sodium toxicity, especially for soils which show lime deficiency.

Further discussion of the entrance of ions into plants may possibly be left to texts of plant biophysics.

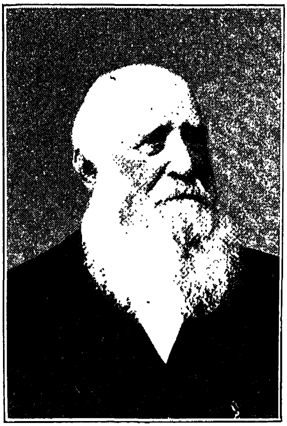
XIX. *Absorption of Organic Constituents of Soils*

It has been shown that plant roots are capable of taking up sugars, amino acids, and other soluble organic substances from soils. However, the concentrations of such substances are very low in the normal soil, and such nutrition is of no great importance for higher plants, though it is of primary importance for the growth of soil fungi and bacteria.

CHAPTER II

METABOLISM OF INORGANIC NUTRIENTS

The special functions of mineral elements has been a phase of general interest in plant physiology. Much was accomplished by Sachs and his students in finding the evidences of deficiency of certain elements in plants. Lawes and Gilbert (Fig. 13) at the Rothamsted, England, Experiment Station contributed much to the theory of plant nutrition, and the conservation of soil fertility.



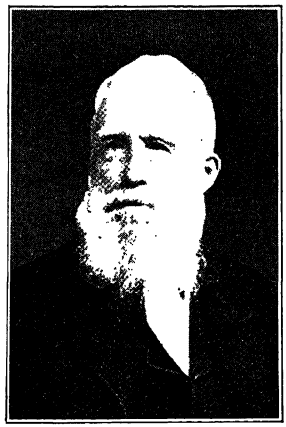


FIG. 13.—Sir Joseph Henry Gilbert, 1817–1901. Sir John Bennet Lawes, 1814–1900.

"For example, it is probable that fungi generally derive nitrogen from organic nitrogen; and in the case of those of fairy rings there can be little doubt that they take up from the soil organic nitrogen which is not available to the meadow plants, and that on their decay their nitrogen becomes available to the associated herbage. Then in the case of the fungus mantle, observed by Frank on the roots of certain trees, it may be supposed that the fungus takes up organic nitrogen and so becomes the medium of the supply of the soil nitrogen to the plant. More pertinent still is the action of the nitrifying organisms in rendering the organic nitrogen of the soil and subsoil available to the higher plants. It may well be supposed, therefore, that there may be other cases in which lower organisms may serve the higher, bringing into a more available condition the combined nitrogen already existing, but in a comparatively inert state in soils and subsoils.

"It seems more consistent, both with experimental results and with general views, to suppose that the nodule-bacteria fix free nitrogen within the higher plant, and that the nitrogenous compounds produced are absorbed and utilized by the plant. In other words, there does not seem to be any evidence that the higher chlorophyllous plant itself fixes free nitrogen, or that the fixation takes place within the soil; but it is much more probable that the lower organisms fix the free nitrogen. If this should eventually be established, we have to recognize a new power of living organisms—that of assimilating an elementary substance."

The Sources of the Nitrogen of Our Leguminous Crops. Journal of the Royal Agricultural Society of England, 52:657–702. 1891.

From the chemical analyses of a soil, it may seem to have an abundance of all elements. Yet certain elements may be in insoluble form or unavailable to plants. It is best to determine the mineral deficiency of soils by applications of the fertilizers directly to the soil.

I. *Potassium*

Potassium occurs in a great many rocks and is a constituent of many of the complex silicates which make up a great part of the inorganic substances of the soil. It is more abundant in igneous rocks generally than in sedimentary rocks, with the exception of sedimentary rocks which contain the remains of the FORAMINIFERA. The potassium content of the silicates of the earth's crust ranges from 0.27% to 11.04%, granites and gneisses contain from 3.3% to 3.5%, limestone and residual clays contain 0.29% to 0.80%. By the weathering of igneous rocks the potassium compounds are rendered more soluble. When a clay soil has had time to form, the potassium will be adsorbed and held by it. Potassium is quite generally distributed over the earth's surface and also in the different layers of soils. In the third nine inches of unfertilized soil at the Rothamsted experimental plots there was more of this element than in the second nine inches. The return to the soil of vegetable remains helps to maintain the supply, especially of such vegetable remains as the stalks of sugar-cane which are very high in potassium content. Potassium salts are contained in considerable abundance in the ash of kelps, because these algæ have the ability of concentrating potassium by absorption from sea-water. Potassium salts are recovered from the flue gases of cement works by precipitation. The alkali lakes of the western part of the United States frequently yield profitable quantities of potassium salts. There are extensive deposits of kainite, crude potassium magnesium chlor-sulphate, at Stassfurt in Germany, and these beds have been the source of much of the world's supply of potassium. Similar deposits also are rather extensive in Texas. Potassium is one of the more important elements in plant nutrition and is liable to be a limiting factor in crop yields. It is especially needed by crops which are grown for sugar production.

Potassium must be in solution before it can be absorbed. It enters the plant at least from the ionic condition, if not as independent K^+ ion.

Within the plant we have no evidence that potassium exists other than in the ionic condition or as fairly soluble salts. Perhaps the least soluble potassium compound in plants is the bitartrate. There are no organic compounds of potassium known in plants other than the salts of organic acids. It has been stated that no potassium is present in the

cell nucleus, although its absolute absence therefrom may be difficult to prove.

When potassium is deficient in the soil or culture solution, the leaves of grass plants and tomatoes (Fig. 14) show a light green color. Potatoes and oranges show a bronzed appearance of the leaves. Evidently there is some interference with the photosynthetic process when potassium is lacking. There is a decrease in the yield of carbohydrate which shows up even before there is a limitation of photosynthesizing surface. The chloroplast does not give any visible cause for the decreased photosynthesis. It should be remembered in this connection that potassium is a radioactive element and for this reason may function in the transformation

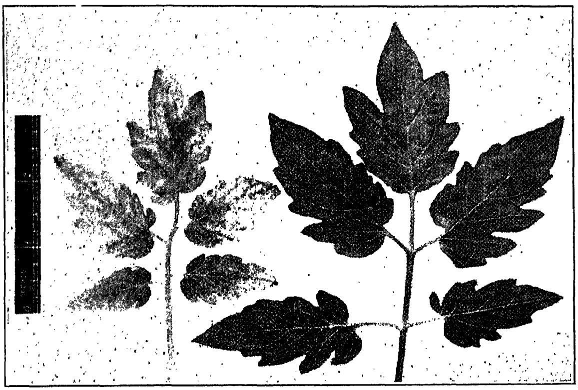


FIG. 14.—Potassium required by tomato plants. Left, leaf showing effects of potassium deficiency.

of radiant energy. In field plots in which there is potassium deficiency there is stunted growth and seeds are sterile. The yield of tubers, bulbs, and other starch or sugar storage organs is especially decreased. Legumes suffer from potassium shortage almost as much as the typical carbohydrate-forming crops. The leaves of alfalfa or clover may show light-colored or white spots in the center of each leaflet when potassium is deficient. Leaf scorch and defoliation develop in apple trees when the potassium supply is deficient. Tobacco leaves show light-colored chlorotic areas. In extreme potassium starvation, plants may fail to reach maturity.

Potassium is abundant in the apices of the plant and in rapidly growing regions in general. It is commonly withdrawn from the older leaves and older plant parts and transported to growing regions. There is but

little potassium in seeds. Potassium is supposed to be necessary for the synthesis of proteins of the protoplasm, but in what manner it functions in this regard has not been explained. Potassium counteracts the effects of calcium on cell colloids and tends to maintain a more fluid condition in the protoplasm. It is said to be concerned with mitotic division of cells; probably it aids in the migration of the chromosomes along the spindle fibers by maintaining a fluid condition in the protoplasm. Potassium favors the accumulation of fats, possibly by its effect in the stimulation of photosynthesis. Potassium increases growth, and this, combined with better carbohydrate supply when it is abundant for cell wall formation, may account for the greater resistance of plants to fungous diseases when the potassium supply is abundant. Potassium may be replaced by sodium only to a minor degree, probably only in so far as the sodium spares the potassium needed in the assimilating surfaces by replacing it elsewhere. Sodium delays the appearance of potassium starvation.

II. *Sodium*

Sodium can hardly be said to have a distinct function in plant nutrition, since it is not required to any considerable extent. Soluble salts of sodium are taken up by halophytes from saline soils in great quantities. The plant sap may be practically a saturated sodium chloride solution. In this case it serves to increase the osmotic concentration and to increase the ability of the plants to hold water against the evaporating power of the air. The addition of salt to the soil tends to make the water requirement less per gram of dry weight laid down by the plant. The effect of sodium on cell colloids is essentially the same as that of potassium. Most plants cannot tolerate very high concentrations of sodium chloride in the soil. But certain groups of halophytes are adapted to it.

III. *Ammonium*

Ammonium ions in the soil are absorbed and used by the plant in the synthesis of protein. Salts of ammonium are important as fertilizer constituents. As a gas in the air ammonia is highly toxic, much more toxic than it should be from the alkaline reaction which it produces in the cell. Gaseous ammonia stimulates the overgrowth of tissues into hyperplasias if the concentration is very low.

IV. *Phosphorus*

Phosphorus occurs in sedimentary rocks as a residuary constituent of the shells of mollusks and of the bones of animals. There are rather

widespread deposits of calcium phosphate derived from the activities of marine animals. Phosphorus occurs also as the mineral apatite in extensive deposits. Both of these minerals are relatively insoluble in water but may be made soluble by treatment with sulphuric acid to form the monobasic and dibasic phosphates as in the manufacture of superphosphate. Phosphorus becomes available also from the decomposition of the constituents of the nuclei of plants and from phosphatides. Granite and gneiss contain from 0.04% to 0.21% of phosphorus. Limestones and residual clays contain from 1.11% to 1.33%. Phosphorus is most liable to be a limiting element in plant nutrition, for although it is rather widespread in soils, the quantity is limited, and there is not much movement of phosphorus from depths of the soil on account of its strong adsorption by soil colloids. Phosphorus enters into combination with aluminium to form insoluble compounds. Part of the beneficial effects of applications of phosphates is due to precipitation of aluminium in the soil, thus removing its toxic action. Phosphorus remains in the plant always in the fully oxidized form of phosphate, although it is combined with organic groups through oxygen linkage. All other forms of phosphorus are poisonous to plants.

The principal use by the plant of phosphorus is in the synthesis of nucleoproteins, and of phosphatides of which it is a constituent. Phosphorus also functions in respiration as a coenzyme of zymase. Probably it aids respiratory processes and the interconversion of hexoses through its action in the formation of hexose phosphates. It enters the glycerophosphoric acid of phosphatides. Phosphorus has some obscurely known function in the accumulation of starch and fat. It is especially abundant in fatty seeds, probably as phosphatides, as nucleins, and as magnesium phosphate in the peculiar globoid bodies found in fatty seeds. Under phosphorus deficiency, plants give evidences of difficulty in their ability to digest starch to sugar, although erythrodextrin and cellulose may be produced. In trial plots of cereals where phosphorus is deficient, there is a decreased growth of straw, followed by very poor yield of grain. Tillering of cereals is decreased when phosphate is deficient, and also there is insufficient root development. Possibly the latter is connected with the effect of phosphates in decreasing aluminium toxicity. The growth of the lateral fibrous root system especially is extended by phosphates. The production of anthocyanins after phosphate starvation in apple trees and in barley may be associated with absorption of aluminium. Phosphorus is accumulated in seeds in organic combination mostly. Its presence in abundance favors early ripening of grains.

In the process of mitosis and the formation of the new nuclear materials considerable phosphate is demanded. Cell-division does not occur

properly if phosphate is deficient. Phosphorus cannot be replaced in the nutrient medium by any other element. It hastens ripening, and for this reason is particularly valuable where the growing season is short.

V. *Sulphur*

Sulphur occurs widely distributed over the earth's surface; in fact, soils which show sulphur deficiency are rather rare. The soils produced from the decomposition of lava in Oregon show sulphur deficiency. Deposits of calcium sulphate, gypsum, are widespread as are also the sulphates of magnesium and alkali metals. Forms of sulphur in all conditions of oxidation or reduction are used in the plant kingdom. The higher plants use only sulphates, the other forms being toxic if present in more than traces in the soil. Hydrogen sulphide is oxidized as a source of energy by sulphur bacteria such as *Beggiatoa*. The end product of such oxidation may be free sulphur, as produced by Phormidia, or sulphates. Frequently, organisms which oxidize hydrogen sulphide to sulphate live in symbiosis with organisms which obtain their oxygen from the sulphates produced by the metabolic activities of the first group. Certain organisms can oxidize thiosulphates. Sulphites are generally quite poisonous to higher plants and to fungi. Sulphur dioxide is used for sterilization purposes. It also prevents the browning of fruits and vegetables by oxidation during the process of drying. The action of oxidases is prevented by sulphur treatment. This gives a clue as to the nature of the toxic action of sulphur dioxide fumes on fungi and bacteria.

Sulphur is a constituent of two amino acids commonly found in proteins, cystine and cysteine. The dipeptide glutamylcysteine, glutathione, is of great importance in respiration as an oxygen carrier. Sulphur is a constituent of volatile oils of the RANUNCULACEÆ, particularly of the BRASSICACEÆ. It is present in white mustard (*Sinapis alba*) as the glucoside sinigrin, which on hydrolysis yields allyl isothiocyanate, which is present also in onion oil with allyl sulphide. There are also other odoriferous sulphur compounds found in plants. The ease with which sulphur compounds are oxidized or reduced by plants indicates that sulphur may serve as an oxygen carrier. Sulphur cannot be replaced in plant nutrition by any other element. The demand for sulphur by plants is low, and it is difficult to exclude from the atmosphere quantities which would perhaps suffice for nutrition. The volatile oil content of onions is decreased when sulphur is kept at a low concentration. This indicates that in a condition of sulphur deficiency the sulphur is used for cysteine and cystine formation at the expense of the sulphur-containing volatile oils. Sulphur added to the soil aids in the solution of other soil constituents

and may be beneficial for this reason. The omission of sulphur from culture solutions causes yellowing and brown spotting of the leaves and brown discoloration of the roots.

Sulphur is found throughout the cell in the masked condition as a constituent of the amino acids mentioned above. Sulphate ion also occurs in cells; in fact, the concentration of sulphate ion in the vacuolar sap may be rather high in certain halophytes of the Arizona desert.

VI. *Iron*

Iron is one of the most abundant elements in the earth's crust. It is widely distributed, and if deficient in soils this is due to insolubility of the iron present, rather than to an actual absence of iron. Iron is present as the sulphide in pyrites and is a constituent of most rocks whether igneous or sedimentary. Iron compounds in the soil are rendered soluble by increased acidity, and this may lead to iron toxicity in very acid soils. In maize grown in acid soil there may be a deposit of Fe_2O_3 in the vascular tract sufficient to interfere with translocation. Firing of the leaves may be observed under such conditions. Where the oxygen supply is good and the content of organic matter in the soil is not excessive, the iron will be in the ferric condition, which is by no means as toxic as ferrous iron is to higher plants. Certain iron bacteria (*Citromyces*) have the ability to oxidize ferrous iron to the ferric condition with a gain of energy from the process. Probably organisms of this type have been of importance in the geologic past in building up the extensive deposits of bog iron ore such as occur in Minnesota.

Plants grown in culture solutions which are deficient in iron show a lack of chlorophyl early in their growth. The cholorosis may be removed by the addition of traces of ferric ion. This gives a clue to one of the most important functions of iron, that of catalyst in the formation of chlorophyl. Iron does not enter into the composition of the chlorophyl molecule, but it is necessary for chlorophyl synthesis. Possibly iron enters into the formation of some chlorophyl precursor. But since the method of action of iron in this regard is unknown, we may describe our ignorance perfectly by saying that it is a catalyst in the process of chlorophyl formation. Iron enters into the molecule of the hematin of animals which has a structure much like chlorophyl and which yields similar units on disruption of the molecule. In hematin of the hemoglobin of blood, iron is an oxygen carrier. It probably acts as an oxygen carrier also in plants. The presence of iron hastens the oxidation of a great many organic substances of the cell. Iron is found rather abundantly in the cell nucleus and here we usually find a high rate of oxidation.

Living protoplasm contains .01% to .001% of iron, a quantity which

is more than sufficient to carry the oxygen used in respiration. This quantity of iron cannot be withheld from cells without stopping growth. If growth occurs in a nutrient solution free from iron, growth stops when the iron contained in the seed has been translocated. If iron is then added, the growth is renewed. The speed of oxidation can be increased by the addition of iron, in some cases, the respiration being proportional to the iron content of the protoplast.

Iron forms a compound with cysteine which is auto-oxidizable, and this may be of great importance in cell respiration. The iron of chromatin is in the masked condition, that is, it does not give the reactions of ferrous or ferric ions. Evidently the iron of the chromatin is in organic combination, yet in what chemical compounds cannot be stated. Chromatin is especially abundant in actively dividing cells of plants. Chromatin probably never leaves the chromosome, so that we are not concerned with the translocation of this type of organic iron compound. It has been suggested that there are more simple organic iron compounds in plants similar to those of animals.

VII. *Manganese*

The properties of manganese are very similar to those of iron, and manganese frequently is found in iron-bearing rocks. The physiological function of manganese in the cell is as an oxygen carrier in oxidations. Manganese increases the oxidation of substances by oxidase action, and, in fact, oxidases may be merely manganese compounds. Probably the thermostable peroxidase is merely manganese which owes its high activity to adsorption upon other protoplasmic constituents. Manganese may be absorbed in quantity by certain trees to such an extent as to render the wood difficulty combustible. Manganese absorption leads to a chlorotic condition in certain plants.

VIII. *Calcium*

Calcium is a very important element both in soils and in plant nutrition on account of its multiple rôle. Calcium is found as a constituent of double silicates in residual clays (2.70% Ca); in granite and gneiss (1.32%–3.17% Ca); in silicate rocks (11.24% Ca); and in limestones (31.90% Ca). Its total absence from soils occurs very rarely. Quartz sands have but little lime. However, the quantity of lime in the soil is frequently deficient. Over the whole eastern part of the United States it is usually found that applications of lime are beneficial to virgin soil. The addition of calcium carbonate or hydroxide to the soil neutralizes the acidity, whatever its cause may be. This leads to flocculation of the soil colloids. The soil becomes more permeable to oxygen and easier to plow. Zeolites are precipitated, and clays are thrown out of suspension by additions of cal-

cium carbonate or bicarbonate. If the calcium is washed out of the soil, the soil colloids may be deflocculated again and form sticky impenetrable masses. Sodium and potassium ions antagonize calcium ion in its action of flocculating soil colloids. Gumbo soils are high in salts of the alkalies. Their fertility may be increased by addition of lime. Sandy soils demand less calcium than clay soils. Soils high in organic matter require more lime than soils with little organic matter. Bog soils of eastern United States are especially liable to show calcium deficiency. Calcium neutralizes acids in the soil whether they are organic acids produced by the partial oxidation of plant residues or inorganic acids produced from smelter fumes or from the efflux of mines.

The flocculation of clays by calcium leads to a more porous structure, which favors the entrance of oxygen and oxidation processes in the soil, which lead to the formation of nitrates from ammonium salts. Nitrogen fixation in the flocculated soil is aided because the penetration of nitrogen gas is better and also because a favorable neutral medium is given for the growth of nitrogen-fixing bacteria of the soil. Calcium has an important rôle in soil economy.

The functions of calcium within the plant are manifoid. As in soils, so in cells, it serves to neutralize acids. Oxalic acid frequently produced by the incomplete oxidation of carbohydrate or protein under conditions of poor oxygen supply is precipitated in insoluble form in specialized cells of leaves. These cystoliths are formed in leaves quite commonly, and deposits of calcium oxalate occur along the tracheæ in stems, forming the crystal fibers of such woods as elm. Malic, tartaric, and other organic acids may be precipitated from solution in cells by combination with calcium. The presence of calcium in culture solutions increases root and leaf development. Roots do not grow well without it. Leaves show brown spotted areas and the old leaves die, while new leaves are mottled and chlorotic. Plants containing chlorophyl generally contain much more calcium than plants of the same families which do not possess chlorophyl. The calcium content increases in leaves as they become older. The cell wall constituents are rendered more firm by an abundance of calcium. This may be partly due to the formation of calcium pectate in the middle lamella, since calcium pectate is a stiffer gel than sodium or potassium pectates. Roots of plants grown in abundance of calcium are noticeably stiffer than those grown in solutions of potassium or sodium. Possibly, also, calcium enters into combination with cellulose of the cell wall and with the acids of cutin and suberin. Calcium seems to be of importance in the regulation of cell permeability. It antagonizes the action of Na^+ and K^+ which tend to increase permeability. Possibly this is due to the formation of calcium soaps with lipoid substances of the cell

surface. The absence of calcium is accompanied by the formation of much cutin on the epidermis. Possibly this is due to increased mobility of the fats with greater condensation to cutin, when calcium is absent.

Calcium is not demanded for the growth of certain fungi and algæ. But in *Coprinus* little mycelium developed and no fruiting bodies formed when calcium was lacking from the nutrient medium.

IX. *Magnesium*

The action of calcium can be replaced at least in part by other ions, particularly by magnesium. In regions where the dolomitic rocks contain much magnesium, this element may replace calcium in the middle lamella, forming magnesium pectate. Also, magnesium pectate can be found on the surfaces of stigmas of plants in such regions.

FIG. 15.—Soil taken from chlorotic area in field. To the pot on the left 400 lbs. per acre of magnesium sulphate were added. The pot on the right received no magnesium sulphate.

Magnesium is frequently associated with calcium in deposits of dolomitic limestones. Magnesium is not usually a limiting factor in soil fertility, since the demands of the plant for it are low. However, enough magnesium must be present for the formation of chlorophyl, for magnesium is a constituent of chlorophyl, being, in fact, a key atom in the structure. The magnesium of chlorophyl is introduced late in the process of chlorophyl synthesis. Its introduction requires light exposure. When magnesium is absent, leaves show white areas, particularly in the vein islets (Fig. 15). Magnesium is found associated with crystalline protein in the globoid and crystalloid bodies of many seeds. The globoid consists of magnesium phosphate. Oil-bearing seeds are especially rich in magnesium. In some manner, magnesium seems to be associated with the formation and accumulation of fats in plants other than through its function in the formation of chlorophyl. Oil globules are not produced in filaments of algæ when magnesium is absent. Magnesium in excessive amounts causes browning of roots and the death of seedlings. This toxic effect is antagonized by calcium.

X. *Silicon*

Silicon is present in the earth in great abundance as complex silicates of many elements and as silicon dioxide. It is probably absorbed as silicate ion. It is absorbed by all plants to a considerable extent. It is especially abundant in grasses, in which it is deposited in the epidermis. The silicate ion on deposition at the cuticular surface evidently loses water and forms a hard deposit of silicon dioxide. This renders the surface hard and difficult to penetrate by fungal hyphæ since silicon dioxide is quite difficultly soluble by organisms. Probably the deposits of silica increase the strength of the cell walls and may prevent the entrance of parasitic fungi through the unbroken surface. That deposits of silica act as protective coverings is shown by the seeds of grasses, Calabar bean, and many other seeds. The seed coats frequently contain a layer of silica which is very hard and probably serves to strengthen the seed coats. Seed coats with high silica and high hemicellulose content are so hard as to turn the edge of a knife.

No organic compounds of silica seem to occur in plants. Silicate seems to lead to greater economy of the use of phosphates by plants, but not by replacing it in organic compounds.

XI. *Aluminium*

Aluminium has a distribution in soils almost as widespread as that of silicon. It is an important constituent of clays and is found in many of the rocks from which soils are formed. Aluminium may occur either as the cation or the anion, depending upon the acidity of the solution. Probably it enters the plant as cation. Aluminium absorption by plants is dependent upon an acid reaction in the soil. When this element is absorbed to excess by maize, nutritional disorders appear in the plants. The young growing areas above the nodes of the stalk show invasion by fungi, and the stalks break over. Deposits of aluminium and iron occur in sufficient quantity in the stalks to block the conducting vessels. Aluminium toxicity of soils can be removed by applications of lime to lower the acidity and thus decrease the concentration of aluminium ion. Phosphates precipitate aluminium at proper acidities.

Aluminium seems to be required by some plants belonging to the Lycopodiales, but for what function is not known. When alum or aluminium chloride is applied to soils in which pink hydrangeas are growing, the pink anthocyanin turns blue. This indicates that aluminium is concerned with the oxidation of the anthocyanins. Probably the similar differences of shades of color shown by other plants, such as the hepaticas, have a similar relationship to aluminium.

The application of soluble aluminium salts to soils or water cultures causes toxic symptoms to appear, owing to aluminium toxicity.

XII. *Boron*

Boron is found as borates in many alkali soils and in alkali lakes of western United States. It is more frequently a limiting factor in fertilizers and soils on account of its presence than on account of a deficiency of it. Boron is used by plants only in small amounts. It seems to be of importance in the conversion and translocation of starch from leaves. It is probably essential for the development of some of the LEGUMINOSÆ. In legumes it seems of importance especially in the formation of vascular elements which run to the root nodules after inoculation. Carbohydrate supply to the bacteria in root nodules may be deficient if the vascular system is not formed, and if there is insufficient conversion of the starch to soluble sugars. One part of boron to one million of culture solution is sufficient. It becomes toxic at concentrations of about 1 5,000.

XIII. *Halogens*

The halogens, chlorine, bromine, iodine, and fluorine are not demanded by plants in more than traces. Increased growth has been reported as following applications of chlorides, iodides, and fluorides in small quantities. The chlorides and iodides are found in rain-water in regions near the sea, in sufficient quantities. Where chlorides are present in the soil, the water requirement is decreased. Large quantities of the halogens are not tolerated when the cation present is also somewhat toxic. Sugar beets seem to produce higher percentages of sugar in soils which contain chlorides.

XIV. *Heavy Metals*

The effects of copper in plant nutrition are of interest mainly because this element is so frequently a constituent of fungicidal and insecticidal applications. Copper is found in the ash of plants and its quantity is increased by the presence of copper carbonate in the soil. In water cultures copper salts become toxic when the concentration is as high as one part in 10,000,000. Algæ may be killed by much lower concentrations in lakes because the copper is accumulated in them from the water; in fact, almost quantitatively removed by them. In low concentrations, copper stimulates growth. It is said to have an oligodynamic effect, but just what that means is not known. Copper can replace magnesium in chlorophyl, and when so substituted makes a light stable compound. Copper may be a catalyst in oxidations, in a manner similar to iron, for in mollusks or other blue-blooded animals, copper of hemocyanin replaces the iron of the hemoglobin of red-blooded animals.

The action of a number of metallic elements is similar to that of copper. Zinc, cobalt, nickel, arsenic, and some similar cations may increase growth and the rate of metabolism when they are present only as a few parts per billion. But all of these elements are very poisonous when the concentration is a few parts per million.

Barium and strontium salts have an action very different from the related calcium or magnesium salts. Strontium is more poisonous than barium to plants. The chloroplasts are rendered inactive by strontium salts.

Salts of the heavy metals, Ag, Hg, Au, Pt, etc., are used as sterilizing agents on account of their toxicity to all groups of plants and animals. They are protoplasm poisons, making insoluble compounds with the cell proteins and thus disorganizing the cell functions and causing death.

XV. *Summary of Nutritional Deficiencies*

The following table (Table 11) taken from Russell's *Soil Conditions and Plant Growth* gives a summary of the nutritional deficiencies indicated by the symptoms shown by various parts of the plant.

TABLE 11

The leaf

Poor leaf growth.		
1. Dwarf plants.	Yellowish colour.	Lack of nitrogen.
	Greyish colour.	Lack of phosphate or potassium.
	Glaucous appearance.	Difficulty of obtaining water, excess of soluble salts, etc.
2. Tall spindly plants.	. . . .	Lack of light near soil. Plants closely packed.
3. Fruit trees.	Bronze purple colour.	Lack of phosphate (apples). Lack of potassium (orange).
	Yellowish, poor growth, premature defoliation.	Competition of grass and other herbage.
Chlorosis or yellowing of leaf.	Uniform all over the leaf.	Lack of iron. Excess of calcium, magnesium, sodium or potassium carbonates. Excess of manganese. Lack of sulphur (tobacco).

TABLE 11 *Continued*

	The leaf	
Chlorosis or yellowing of leaf.	Patchy, spreading from midrib outwards.	Lack of magnesium.
	Mottled.	Lack of calcium.
	Spotty.	Lack of potassium.
	Leaf yellowing and then dying at tip and from edges inwards.	Lack of potassium.
	Leaf yellowing and dying from midrib outwards.	Lack of nitrogen
Patches on leaf.	Brown patches resembling scorch (fruit trees).	Lack of potassium.
	Brown patches chiefly in centre.	Lack of magnesium.
	Brown spots.	Lack of calcium.
Premature defoliation.		Lack of potassium, magnesium.
Rich green leaves and large thick stems.		Large supply of nitrogen.
Dark coloured leaves, tendency to crinkle.		Insufficient potassium in relation to nitrogen.
Patchy appearance of herbage, some dark-green, some lighter.		Acidity of soil.
	Root	
Very stunted.	Acidity. Lack of calcium or phosphate: bad aëration: lack of drainage: clay soil.	
Much fibrous development.	Good aëration: sandy soil.	
	Fruit	
Brilliant red (apples).	Competition of grass.	
Blotchy (tomatoes).	Lack of potassium.	
	Seed	
Delayed ripening.	Excess of water: excess of nitrogen: lack of phosphate.	
Failure to reach maturity.	Great lack of potassium.	

CHAPTER III

CHEMOSYNTHESIS AND THE SPECIAL METABOLISM OF CARBON, NITROGEN, SULPHUR, AND IRON

I. *Chemosynthesis and Carbon Assimilation*

Chemosynthesis is a process of assimilating carbon from carbonates, in which the energy required for the reduction is derived from the oxidation of inorganic substances. It is to be distinguished from photosynthesis, in which the energy is derived from light absorption. The organisms which carry on chemosynthesis are autotrophic. They are of the greatest importance in transformations of substances in soils. The oxidation of reduced forms of nitrogen, sulphur, iron, and hydrogen serves as the source of energy, which is used in the assimilation of the carbonates. The physiology of these organisms is of especial interest and importance, because their metabolism is of such nature as to set them apart from the heterotrophic bacteria. The obligate autotrophic bacteria require the presence in the reduced form of the particular elements which they use in their chemosynthesis. The energy of oxidation of these substances may be their only source of energy for carbon assimilation. Carbonates in solution may be their only source of carbon.

The group of obligate autotrophic bacteria show transition forms which are facultatively autotrophic, and this link serves to connect the chemosynthetic forms with the more abundant heterotrophic bacteria. Depending upon the presence of organic substances in their immediate environs, the type of metabolism of the facultative chemosynthetic forms may change from strict autotrophism to saprophytism. To many of the strictly autotrophic forms, organic substances in more than low concentrations are injurious. The metabolism of the various groups has been most studied in their relationships to soils, since they are of great importance in soil chemistry. But the importance of these organisms as primitive forms in evolution and as formers of extensive deposits of bog iron ore and of sulphur should not be overlooked. We owe to Winogradski and his students a great part of the work on the culture and metabolism of these organisms which oxidize the reduced compounds of sulphur, iron, and nitrogen.

II. *Special Metabolism of Sulphur*

On the basis of their metabolic processes the organisms which oxidize sulphur compounds may be classified into:

1. Those forms which oxidize hydrogen sulphide principally with deposition of elemental sulphur in their cells. The members of this group may be classified further morphologically into (a) filamentous colorless bacteria represented by *Thiothrix* and *Beggiatoa;* (b) non filamentous colorless bacteria, such as *Thiospirillum*, *Thiovulum*, and *Achromatium;* (c) purple sulphur bacteria which contain a purple-red pigment, bacteriopurpurin, and a green pigment, bacteriochlorin. The group (c) has been regarded as beginning the photosynthetic organisms or probably being the connecting link in the evolution of that group. And, (d) those peculiarly adapted organisms, such as *Phormidium*, which live in hot springs only. On account of the absence from these latter organisms of all of the thermolabile enzymes for which tests have been made, it would seem desirable to classify these organisms separately on a physiological basis. Their metabolic processes are certainly carried out in a different manner from those of organisms which live at low temperatures.
2. Those non-filamentous bacteria which oxidize hydrogen sulphide or thiosulphates to free sulphur, the deposit of sulphur occurring outside of the cells. Examples are *Thiobacillus denitrificans* and *Thiobacillus thioparus.*
3. Non-filamentous bacteria which oxidize elemental sulphur very rapidly, but which also can oxidize H_2S and thiosulphates. Only one species has been shown to have this ability, *Thiobacillus thio oxid*

Those organisms which act mainly in the oxidation of H_2S to elemental sulphur, forming deposits in the cells, evidently have also the ability to oxidize elemental sulphur. The reaction for the oxidation of hydrogen sulphide may be written as follows: $2H_2S + O_2 \rightarrow 2H_2O + S_2 +$ 122.2 cal. The sulphur may be oxidized with a further liberation of energy as follows: $S_2 + 3O_2 + 2H_2O \rightarrow 2H_2SO_4 + 284.4$ cal. If the supply of H_2S is insufficient, the sulphur contained in the filaments may be further oxidized to sulphates. The deposited elemental sulphur must then be regarded as a substance deposited as an energy reserve for use in case of starvation. When their free sulphur has been used up, the cells die. The oxidation of sulphur to sulphate demands that there shall be present substances for the neutralization of the sulphuric acid which otherwise would accumulate. Calcium carbonate and bicarbonate are present in the medium at the acidities tolerated by this group, and the calcium serves to neutralize the sulphuric acid. The carbonate goes to form the organic substances of the protoplasm. In the case of oxidation of H_2S to sulphur only, such neutralization is not necessary. The stage to which the oxidation proceeds is evidently determined by the oxidation potential of the medium. These organisms tolerate only traces of soluble organic substances. They do not use any elaborated carbon compounds as a source of energy in their nutrition. In the reduction of one gram of carbon from carbonates these organisms use the energy liberated from the oxidation of 8 to 10 grams of sulphur. Sulphur external to the cells cannot be utilized. The oxidation of sulphur compounds is evidently carried on

entirely within the cells, that is, the process is intracellular oxidation, as evidenced by the deposition and removal of sulphur. The nitrogen supply of these organisms is derived from ammonium salts alone.

Evidently extracellular oxidation of H_2S occurs in those organisms which cause the deposit of sulphur outside of the filaments. Otherwise we should have difficulty in accounting for the transport of elemental sulphur which is insoluble in an aqueous medium, from the inside to the outside of the cells. The sulphur is deposited at some distance from the bacterial colony. These forms also oxidize thiosulphates to free sulphur. It has been suggested that the sulphur deposited at a distance from the cells is produced by a secondary reaction between thiosulphate in the medium and the tetrathionate produced by the oxidation of part of the thiosulphate, or produced from the oxidation of H_2S by the cells. The reactions may be indicated as follows:

$$)_3 + 6O_2 \rightarrow 4Na_2S_2O_6 \quad \text{sodium dithionate}$$
$$)_3 + 5O_2 \rightarrow 2Na_2S_4O_6 + Na_2SO_4 \quad \text{sodium tetrathionate}$$

These organisms can use calcium sulphide and tetrathionate as well as hydrogen sulphide and thiosulphate. Their carbon source is from carbonate in solution. The nitrogen source may be either ammonium salts or nitrates. The oxygen of nitrates may be used in the oxidations to obtain energy. The group includes both aërobes, and anaërobes which obtain their oxygen from nitrates. The nitrate may be reduced to elemental nitrogen. *Thiobacillus denitrificans*, a member of this group, is of widespread occurrence in soils. It is not injured by organic substances which it can use, but is facultatively autotrophic. It produces elemental nitrogen from nitrates by removal of the oxygen, but also can function by using atmospheric oxygen. It is to be regarded as a connecting link between the sulphur-oxidizing bacteria and those which produce denitrification. Its sulphur and nitrogen transformations may be represented as follows:

5

$$5Na_2S_2O_3 + 8KNO_3 + 2NaHCO_3 \rightarrow 6Na_2SO_4 + 4K_2SO_4 + 2CO_2 + 4N_2 + H_2O$$

Of great importance in the oxidation of elemental sulphur in the soil is the single organism *Thiobacillus thio-oxidans*. Evidently this organism brings about an extracellular oxidation of the sulphur, for it is difficult to picture a mechanism for the penetration of free sulphur into the cells. This organism is very resistant to the sulphuric acid which it produces. The medium may become as acid as pH 0.58. The organism is strictly aërobic and evidently requires a high rate of oxygen supply, for its growth is confined to the surface of culture media, and aëration favors its develop-

ment. The carbon supply is from H_2CO_3. At the acidities maintained by the cultures, scarcely any free carbonate would exist. Calcium carbonate is transformed to sulphate and precipitates. The presence of organic matter is not injurious, in fact some stimulation of growth occurs from additions of sugars, ethyl alcohol, and glycerin, without these substances being used in the carbon economy of the organism. Nitrogenous substances must not be present in concentration more than a few tenths of one per cent, for they have an injurious effect on this organism. The energy liberation by *Thiobacillus thio-oxidans* is very considerable, since one mole of sulphur on oxidation to sulphuric acid yields 142.2 calories. About $6^2/_3$% of the energy liberated by the oxidation of sulphur is used in the chemosynthesis of carbon.

The oxygen of sulphates can be used by certain bacteria which are present to great depths in the soil. Such organisms as *Microspira desulfuricans* require the absence of free oxygen. They are obligate anaërobes. They must have a supply of elaborated carbon compounds which they oxidize with the oxygen derived from sulphates. Their activity produces H_2S, which may produce black deposits with iron compounds. The color of black muds, as in the Black Sea, is due frequently to their action. Some facultative anaërobic fungi of the genus *Actinomyces* are common in soils and are of importance in the sulphur cycle in soils. These sulphur-reducing organisms live in symbiosis with sulphur oxidizing organisms. The reduction of sulphuric acid to hydrogen sulphide consumes considerable energy. The reaction may be represented as follows:

$$H_2SO_4 + 135 \text{ cal.} = H_2S + 2O_2$$

The energy required comes from the oxidation of carbon compounds. Evidently the efficiency of such oxidations is low, a major part, probably 80% of the energy liberated in the oxidation, being required for the reduction of sulphates.

The decomposition of sulphur compounds of the protoplast to form hydrogen sulphide may be produced by a great many putrefactive bacteria. Among the obligate anaërobes *Bacillus putrificus* and *Bacillus sporogenes* are important in the production of H_2S. The very common bacterium from the colon, *Bacillus coli*, is responsible for the formation of much of the hydrogen sulphide of feces. This organism is a facultative anaërobe, as are also *Bacterium vulgare* and *Staphylococcus pyogenes aureus* which also produce H_2S. Evidently a great many organisms produce H_2S in putrefactive processes. The quantity of H_2S produced depends usually upon the oxidation potential of the putrefying mass. The sulphate-forming organisms are present universally.

Much of the H_2S in putrefactions comes from proteins which contain

cystein and cystine. Taurine of the bile is a source of H_2S in feces. Mercaptans, either ethyl mercaptan, C_2H_5HS, or methyl mercaptan, CH_3HS, may accompany the hydrogen sulphide in feces. These substances are also found in certain plants and may be produced in a similar manner under reducing conditions.

III. *Special Metabolism of Iron*

Only a few bacteria are known to require reduced iron compounds for carrying on their carbon assimilation. A larger number of bacteria can use the oxidation of iron as a source of energy but live mainly on organic substances. A still greater number of organisms may merely adsorb or accumulate iron oxide in deposits on the surface of the cells.

The organisms which are restricted to the oxidation of iron for their energy source, such as certain species of *Spirophyllum* and *Leptothrix*, will grow only in solutions which contain iron in the ferric condition. Ferrous carbonate is oxidized to ferric hydroxide with a liberation of energy, $2FeCO_3 + H_2O \rightarrow Fe_2\ (OH)_6 + 2CO_2 + 29.8$ calories. The carbon dioxide involved in this equation may partly be assimilated to form the organic substance of the protoplast. But the gain of energy in the process is very little; the bacteria oxidize a great quantity of iron to obtain their energy.

Certain species of *Leptothrix* can utilize either ferrous carbonate or soluble organic iron salts. *Leptothrix* oxidizes the organic part of the compounds and causes the precipitation of ferric hydroxide. Such organisms may be regarded as facultative autotrophs.

Various strains of iron bacteria are capable of oxidizing manganese compounds in a similar manner and they use the energy so derived for chemosynthesis.

IV. *Special Metabolism of Nitrogen*

1. NITROGEN FIXATION

The most abundant source of nitrogen, the atmospheric nitrogen, is not directly available to higher plants for use in their synthesis. In fact, the metabolism of most organisms leads to nitrogen losses. Except for the small amounts of nitrogen fixed by artificial means, the restoration of nitrogen to the soil is accomplished by a small number of bacteria which have the ability of nitrogen fixation. These organisms may add to the soil quantities of nitrogen which, if it were necessary to produce it by the artificial synthesis at command, would involve excessive expense. The nitrogen fixation by organisms is enormously greater than all of our nitrogen fixation by artificial synthesis. A crop of legumes under favora-

ble conditions may add as much as 200 pounds of nitrogen per acre to the soil per year.

The reduction of nitrogen by hydrogen to form ammonia, as in nitrogen fixation, evolves energy. Since the reaction is exothermic, it should proceed without the use of energy from other sources. The reaction may be represented as follows: $\underset{10\%}{N_2 + 3H_2} \rightleftarrows \underset{90\%}{2NH_3} + 24$ Cal. The reaction is easily reversible. At ordinary temperatures only 10% of the nitrogen is uncombined. At higher temperatures the proportion of uncombined nitrogen is increased, since raising the temperature tends to reverse the exothermic reaction. If the cells of bacteria produce hydrogen in a reactive condition, it may combine with nitrogen with a yield of energy in the process. There is no reason why this energy may not be used just as well as the energy liberated by the oxidation of sulphur or iron. The combination of molecular nitrogen and molecular hydrogen at ordinary temperatures proceeds very slowly. The energy liberation is small. A catalyst increases the rate of the reaction to establish equilibrium. It is possible that there exist in cells catalysts which are capable of speeding up this reaction, for cells have similar catalysts for combining hydrogen with oxygen. Possibly also in the metabolism of the cells, hydrogen may be produced in the atomic state in which it may react with nitrogen. The energy derived from combination with atomic hydrogen should be greater than that liberated from combination with gaseous hydrogen, since there is considerable energy liberated in the formation of gaseous hydrogen from atomic hydrogen. Furthermore, the energy liberated in going from gaseous nitrogen to form the amino group of amino acids may be still greater than that indicated in the synthesis of ammonia, since it probably involves the amination of an hydroxy acid. Since only energy liberation is concerned, there is no difficulty from the thermodynamics of the reaction. But the conditions would need to be such that atomic hydrogen, or at least molecular hydrogen, might be formed. The sulphur and iron bacteria which are obligate autotrophs require reduced substances for their proper functioning. They grow in media containing hydrogen sulphide or ferrous iron, etc. The oxidation potential of such media is such as to produce the reducing conditions under which the hydrogen may be produced for nitrogen fixation. It is known that certain bacteria which produce hydrogen under anaërobic conditions also fix atmospheric nitrogen. The hydrogen required for the fixation of nitrogen may come from such reactions as the production of butyric acid and hydrogen from the butyric fermentation of hexoses. Whether the first reaction in nitrogen fixation is the production of ammonia or whether the amino acids of bacterial proteins are formed directly is of little importance. Perhaps we should be more correct

in physiology if we should consider that reactions which may be stopped at definite chemical compounds do not necessarily need to do so in a medium where there are continually so many free bonds and so many chemical transformations involving ionic exchange. The fact that a series of chemical substances can be produced in a series of stages *in vitro*, or even *in vivo*, by causing abnormal conditions, is no argument that they are formed as such in organisms. We have gained much information recently on the importance of free valencies and labile conditions in the organism. Steps in the reduction of nitrogen involving intermediate compounds have been outlined as follows: $N \equiv N \xrightarrow{2H}$

2H

If the fixation of atmospheric nitrogen by autotrophic bacteria is dependent upon essentially anaërobic conditions in the cell, it may be perceived that higher plants necessarily must have lost this ability when they began photosynthesis and the liberation of oxygen thereby.

The heterotrophic bacteria which fix atmospheric nitrogen may be grouped into:

1. Free living soil bacteria, such as: (a) Aërobic—*Azotobacter chroöcoccum*, and *A. agile; Bacillus asterosporus*. and *B. ellenbachiensis*, *Bacterium aërogenes*, *Radiobacter*. (b) Anaërobic—*Closteridium pastorianum* (*Bacillus amylobacter*).
2. Symbiotic organisms living in tubercles—*Bacillus radicicola*, *Bacterium rubiacearum*.

The free living heterotrophic bacteria obtain energy from the oxidation of organic substances of the soil, both carbon and nitrogen compounds. Their essentially heterotrophic nature is shown by the fact that if the supply of fixed nitrogen is sufficient they do not fix atmospheric nitrogen or not to so great an extent. Thus when no nitrogen source was available other than atmospheric nitrogen, a culture of *Azotobacter*, obtaining its energy from the oxidation of 4% glucose solution, fixed 24.68 mg. of nitrogen per liter in 15 days. When more than 0.6 gm. of ammonia or nitrate nitrogen was added per 100 grams of glucose, nitrogen fixation ceased entirely. Also, the presence of usable organic material decreased the efficiency of nitrogen fixation per gram of substance oxidized. For instance, in increasing the concentration of mannitol in the culture medium, the efficiency of nitrogen fixation by *Azotobacter* decreased as shown by the following data:

Mannite, per cent....................	0.1	0.2	0.5	1.0	1.5
Nitrogen fixed per gm. of mannite, in mgs.	10.5	8.3	6.4	4.68	3.22

Most of the heterotrophic non-symbiotic nitrogen-fixing bacteria are aërobes. The process of nitrogen fixation is favored by tillage and by a porous structure of the soil. The aërobes oxidize the soluble organic

substances, carbohydrates, alcohols, etc., produced from the plant and animal remains with the production of carbon dioxide and some organic acids. It is desirable to have sufficient calcium carbonate present for the neutralization of the acids, for these organisms grow best in neutral media (Fig. 16). *Closteridium pastorianum* is an anaërobe and is respon-

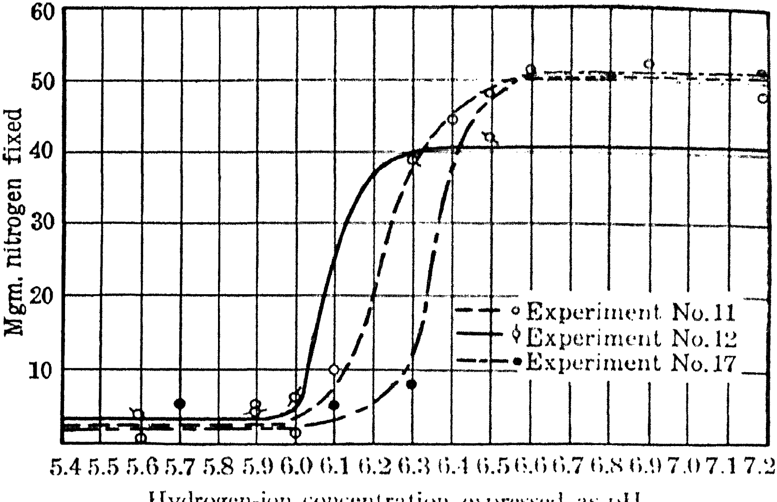


FIG. 16. Influence of reaction of medium upon n fixation by *Azotobacter* (fro and Bat

sible for the nitrogen fixation under conditions in which the aërobes cannot function. *Closteridium* carries on the fermentation of organic substances producing organic acids, chiefly butyric and acetic acids, in the process. On the basis of the organic matter transformed, *Closteridium* is not as efficient a nitrogen fixer as the aërobic *Azotobacter*, but the energy liberation by *Closteridium* is much less on account of the inefficiency of anaërobic oxidations in energy liberation. The reducing conditions under which *Closteridium* operates give it actually a higher efficiency in nitrogen fixation when the carbon dioxide produced is used as the measure of energy liberation. The gas produced by the fermentations of *Closteridium* consists of 40% CO_2 and 51% H_2. This gives a clear indication of the highly reducing conditions in the cells of this organism. Some bacteria other than *Closteridium* which carry on the butyric fermentation also fix atmospheric nitrogen.

The nitrogen compounds brought into the soil by free living bacteria

become available to crop plants upon death and the hydrolysis of the bacterial proteins in the soil.

The symbiotic bacteria found in nodules are highly specialized, being adapted to a limited range of host plants. They may form nodules on the leaves of plants belonging to the family of the RUBIACEÆ (*Bacterium rubiacearum*), such as the genera *Pavetta* and *Grumilea*, and to the family MYRSINACEÆ, such as the genus *Ardisia* (*Bacterium foliicola*). In leaf nodules the nitrogen fixing organisms may enter the host through the stomata of the leaf, as in the case of *Pavetta*. Or they may live in intimate hereditary symbiotic relation with the host plant, penetrating throughout its tissue and entering the embryo sac, so that the embryo becomes inoculated. When the seed germinates, the symbiotic bacteria are carried out with the growing tips. The bacteria in leaves live in the intercellular spaces. Evidently their activity leads to the production of substances which stimulate hypertrophy of the cells to form the tubercle. From the known effects of ammonia in producing overgrowths of cells, its presence may be suspected of being instrumental in the production of the overgrowth. The leaf nodule formers are aërobic. The leaf nodule formation is limited to a few tropical plants.

The formation of nodules on the roots of plants is more common than the occurrence of nodules on the leaves A symbiotic relationship has been established extensively between the group of the LEGUMINOSÆ and the *Bacillus radicicola*. Nodules are formed also on the roots of *Eleagnus*, *Alnus*, *Ceanothus*, *Podocarpus*, *Cycas*, *Myrica*, and *Casuarina*. In some of these cases there is a symbiotic relationship between *Bacillus radicicola*, certain algæ, and *Azotobacter*. *Actinomyces* has been reported as the symbiotic organism in some root nodules.

The high specificity of the strains of *Bacillus radicicola*, which may be classified into *Rhizobium leguminosum* and *R. radicicolum*, is shown by the ability of the strains to grow only on certain species of the legumes. The strains may be classified with regard to their alternate hosts as follows (Table 12).

TABLE 12

Group I:	Group II:
Trifolium pratense, red clover	*Melilotus alba*, white sweet clover
Trifolium hybridum, alsike clover	*Melilotus officinalis*, yellow sweet clover
Trifolium alexandrinum, berseem clover	*Medicago sativa*, alfalfa
Trifolium incarnatum, crimson clover	*Medicago hispida*, bur clover
Trifolium medium, zigzag, or cow clover	*Medicago lupulina*, black medic, or yellow trefoil
Trifolium repens, white clover	*Trigonella fænumgræcum*, fenugreek

TABLE 12—*Continued*

Group III:
- *Vigna sinensis*, cow-pea
- *Cassia chamaecrista*, partridge pea
- *Arachis hypogea*, peanut
- *Lespedeza striata*, Japan clover
- *Mucuna utilis*, velvet bean
- *Baptisia tinctoria*, wild indigo
- *Desmodium canescens*, tick trefoil
- *Acacia armata*, acacia
- *Genista tinctoria*, dyer's greenweed
- *Phaseolus lunatus*, Lima bean

Group IV:
- *Pisum sativum arvense*, Canada
- *Vicia villosa*, hairy vetch
- *Vicia sativa*, spring vetch
- *Vicia faba*, broad bean
- *Lens esculenta*, lentil
- *Lathyrus latifolius*, sweet pea

Group V:
- *Glycine hispida* (*Soja soja*), soybean

Group VI:
- *Phaseolus vulgaris*, garden bean
- *Phaseolus multiflorus*, scarlet runner bean

Group VII:
- *Lupinus perennis*, lupine
- *Ornithopus sativa*, serradilla

Group VIII:
- *Amphicarpa monoica*, hog peanut

Group IX:
- *Amorpha canescens*, lead plant

Group X:
- *Strophostyles helvola*, trailing wild bean

Group XI:
- *Robinia pseudacacia*, black or common locust

Group XII:
- *Dalea alopecuroides*, woods' clover

Bacillus radicicola may be found in soils in which inoculated plants have been grown. The bacteria in the soil are actively motile. They infect the host plant by entering the young roots usually through root hairs. At first the bacteria seem to be mostly parasitic, and at high soil temperatures the parasitism is emphasized. As the nodule formation proceeds with the growth of the root tissue around the infection spot, the bacteria are changed morphologically. They lose their flagella and are transformed into somewhat filamentous forms called *bacteroids*. The bacteroids are frequently X- or Y-shaped forms. The bacteroids are attenuated in their virulence and evidently better adapted to symbiosis with the host than the original motile forms which infect the root hairs.

The symbiotic nitrogen-fixing organisms derive a supply of organic materials from their host plants. In culture media various carbohydrates may be used as the carbon source. No fixed nitrogen source is demanded since the organisms readily fix atmospheric nitrogen. The presence of fixed nitrogen decreases the nitrogen fixation when the bacteria are grown in culture media. In the tubercle, the nitrogen formed into bacterial protein becomes available to the host plant upon the death of the bac-

terial cell. There has been demonstrated a bacteriophage in the nodules which causes dissolution of the bacteria, making their nitrogen compounds available to the host plant. It has also been stated that there is excretion of nitrogenous substances from the live bacteria in the nodule.

2. DECOMPOSITION OF COMPLEX NITROGEN COMPOUNDS—AMMONIFICATION

When plants or animals die, the nitrogen compounds which they contain go through putrefactive processes which again bring the nitrogen into forms assimilable by plants. The death of the cells does not necessitate the cessation of enzyme activity. Autolysis of the dead cell occurs from the action of the cell's own enzymes. The cell proteins are digested and may be brought into solution by hydrolysis, forming an excellent medium for the growth of bacteria. The bacteria already present in the organism before death may begin putrefaction, but there are always present and distributed by the air large numbers of putrefactive bacteria to complete the decomposition of the proteins. It is scarcely possible for a dead cat to fall by the wayside without its presence becoming known from the products of putrefaction.

The bacteria which bring about protein hydrolysis, such as *Bacillus subtilis*, *Bacillus mycoides*, etc., use the peptides and amino acids in their metabolism. Since a great amount of heat energy is liberated by the growth of these bacteria, the carbon chains of the amino acids are oxidized and various decomposition products are formed, including CO_2, organic acids, alcohols, aldehydes, hydrogen sulphide, ammonia, and amines. The products depend upon the nature of the protein acted upon, upon the kind of organisms producing the decomposition, and upon the environmental conditions. The presence or absence of oxygen and the oxidation potential of the medium are of great importance in determining the nature of the products. When the oxidation potential is low, there is extensive production of reduced compounds, such as ammonia, hydrogen sulphide, amines, butyric acid, etc., which are ill-smelling gaseous products, and also strongly odoriferous phenolic compounds such as indol and skatol are produced.

The products of the decomposition by one bacterium are acted upon by other bacteria, so that toward the end of the decomposition of the mass the products are different from what they were at the beginning of putrefaction. Under natural conditions there is a great mixture of bacteria present.

The aliphatic amino acids are more easily decomposed than the aromatic imino acid compounds. Straight chain amino acids under aërobic conditions are deaminized with the production of fatty acids, alcohols, aldehydes, CO_2, and ammonia.

$$\mathrm{R\,CH\,NH_2COOH + H_2O \rightarrow R\,CHOH\,COOH + NH_3}$$
$$\mathrm{R\,CH\,NH_2COOH + H_2O \rightarrow R\,CH_2OH + CO_2 + NH_3}$$
$$\mathrm{R\,CH\,NH_2COOH + H_2O \rightarrow R\,CHO + HCOOH + NH_3}$$

Alcohols may be produced from such amino acids as leucine by bacteria, yeasts, and other fungi.

The carboxyl group may be removed from the amino acid with formation of amines which on further bacterial action produce ammonia.

$$\mathrm{R\,CH\,NH_2COOH \rightarrow R\,CH_2NH_2 + CO_2}$$
$$\mathrm{R\,CH_2NH_2 + H_2O \rightarrow R\,CH_2OH + NH_3}$$

With extreme reducing conditions under the action of anaërobes, reductive deamination of the amino acid may occur:

$$\mathrm{R\,CH\,NH_2COOH + H_2 \rightarrow R\,CH_2COOH + NH_3}$$
$$\mathrm{R\,CH\,NH_2COOH + H_2 \rightarrow R\,CH_3 + NH_3 + CO_2}$$

The latter reaction leads to the production of methane frequently under the anaërobic conditions existing in bogs.

In the presence of free oxygen, oxidative deamination is carried on by bacteria and fungi:

$$\mathrm{R\,CH\,NH_2COOH + O_2 \rightarrow R\,COOH + NH_3 + CO_2}$$

The bacteria of the soil are very active in protein decomposition *Bacillus mycoides* is one of the most common of soil bacteria, and it is one of the most active organisms in protein decomposition. Other common ammonifiers are: *Bacterium vulgare*, *Bacterium prodigiosum*, *Bacterium fluorescens liquefaciens*, *Bacillus tumescens*, and *Bacterium subtilis*. *Bacillus cereus* is especially important in the cleavage of proteins to amino acids. Certain bacteria, such as *Bacillus coli* and *Bacterium filiformis aerobius*, do not act upon proteins directly, but only upon cleavage products of the proteins. Their functioning is dependent upon the previous action of such bacteria as *Bacillus cereus*.

3. NITRIFICATION—THE OXIDATION OF AMMONIA TO NITRITES

The action of the ammonifying bacteria on protein substances yields a reduced compound, ammonia. This ammonia can be oxidized to nitrites by such bacteria as *Nitrosomonas* and *Nitrosococcus*. These organisms gain energy from this oxidation. They are injured by high concentrations of organic materials, especially if the oxygen supply is poor. The source of carbon for these bacteria is from carbonates. They are aërobic forms, to be found only at the surface of decaying masses, such as manure piles.

Deep layers of manure contain only few nitrite formers. They prefer an alkaline reaction, pH 8.4-9.0, being thus adapted to conditions under which free NH_3 should appear (Fig. 17).

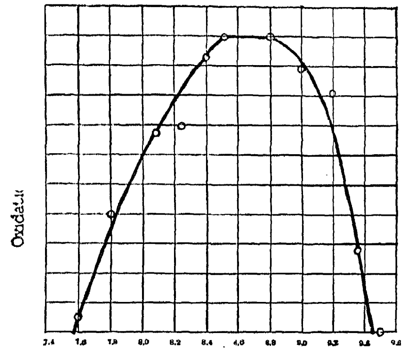
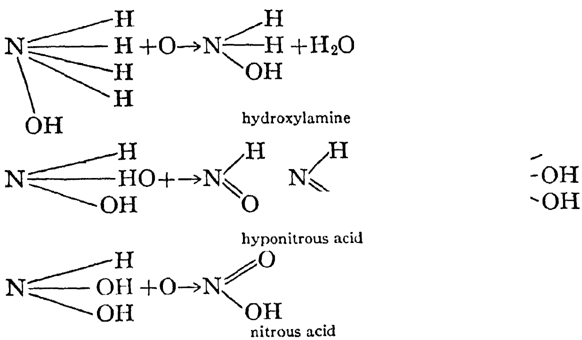


FIG. 17.—Influence of reaction upon the respiration of nitrite-forming bacteria (after Meyerhof).

The oxidation of ammonia probably proceeds through a series of steps, forming first hydroxylamine and then hyponitrous acid from the ammonia as follows:



The end result of the reactions may be represented as follows: $2NH_3 + 3O_2 \rightarrow 2HNO_2 + 2H_2O + 156.8$ calories

These nitrite formers are strictly autotrophic. They do not oxidize organic substances in the medium.

The energy liberated from the oxidation of ammonia is used for the synthesis of carbon compounds from carbon dioxide or carbonates.

4. THE OXIDATION OF NITRITE TO NITRATE

The oxidation of nitrites to nitrates involves energy liberation. This energy is used by nitrate-forming bacteria, such as *Nitrobacter*, in the

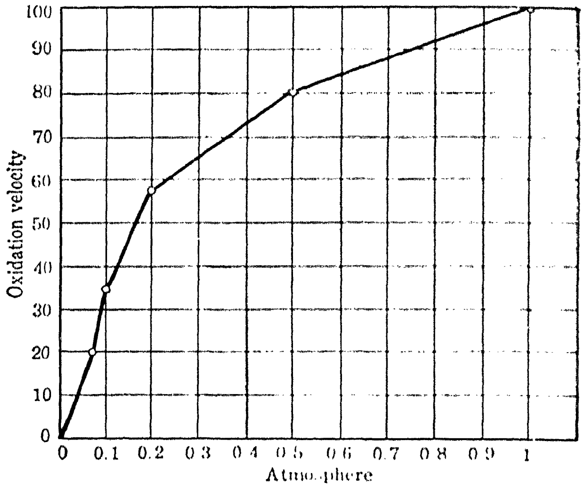


FIG. 18. Influence of oxygen pressure upon the oxidation of nitrite to nitrate (from Meyerhof).

chemosynthesis of carbon compounds. The oxidation reaction may be represented as follows:

$$2HNO_2 + O_2 \rightarrow 2HNO_3 + 37.6 \text{ calories}$$

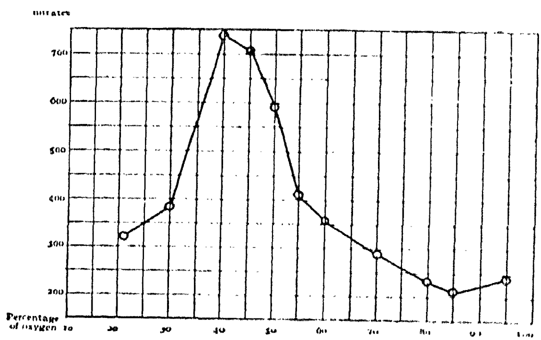


FIG. 19. Influence of oxygen tension upon nitrate formation in the soil (from Plummer).

Free oxygen is required for this process. The organisms bringing about nitrite oxidation are obligate aërobes (Figs. 18, 19). The nitrate formers

do not use any elaborated carbon compounds in the medium for their respiration; in fact, they are injured by organic substances. All of the oxygen absorbed is used in the oxidation of nitrite to nitrate. From five cultures

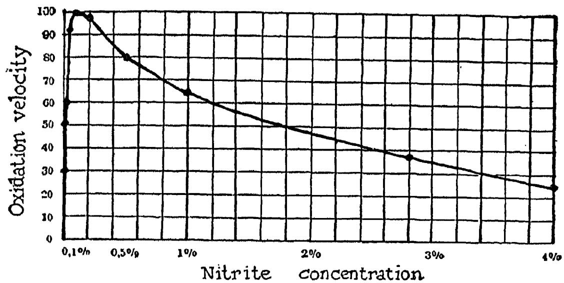


FIG. 20.—Influence of nitrite concentration upon the oxidation velocity of nitrate-forming bacteria (from Meyerhof).

of *Nitrobacter* the average oxygen consumed was 0.295 c. c., while the oxygen required for the nitrate which they formed from nitrite was 0.298 c. c.

The only source of carbon assimilated by *Nitrobacter* is the carbonate. About 135 milligrams of nitrogen is oxidized from the nitrite to the nitrate

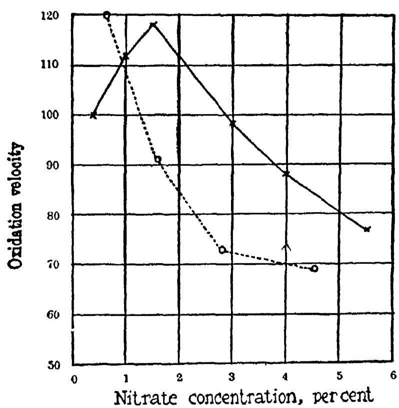


FIG. 21.—Influence of nitrate concentration upon the growth (- - - - -) and respiration (————) of nitrate-forming bacteria (from Meyerhof).

condition for each milligram of carbon dioxide assimilated. For the synthesis of carbon compounds alone, the organism uses only about 5.2% of the energy which it liberates in the oxidation process.

The oxidation of the nitrate formers is inhibited by the presence either of nitrite or nitrate, except in low concentration, as shown by the graphs in Figs. 20, 21.

The oxygen supply of the soil is influenced greatly by the porosity of the soil. When the soil is 50–80% of saturation, the respiration of nitrate-forming bacteria is most active. In water-logged soil the oxidation of nitrite is decreased until it falls almost to zero in a fully saturated soil (Fig. 22).

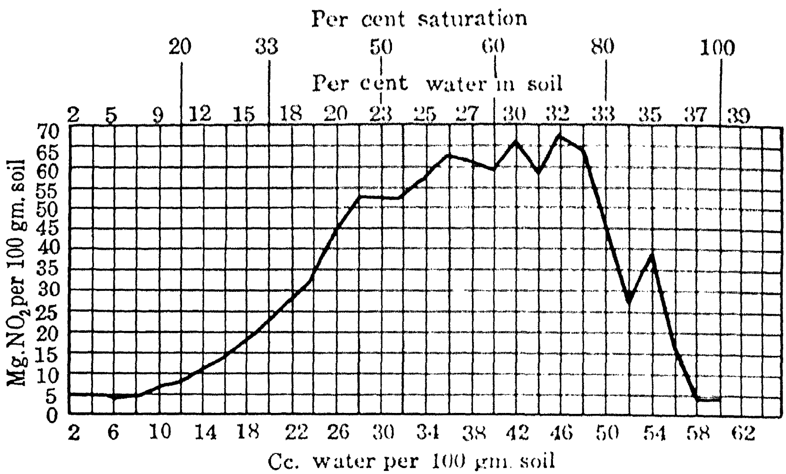


FIG. 22. Influence of moisture content upon nitrate production in the soil (from Gainey).

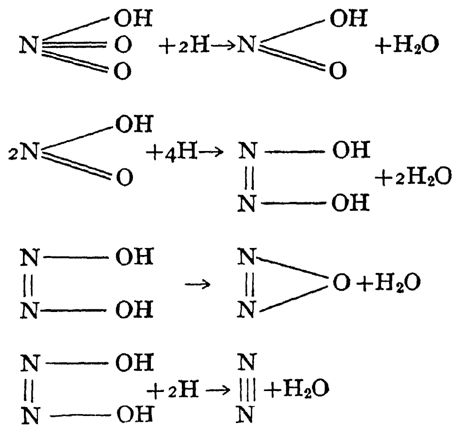
5. DENITRIFICATION

Of great importance in soil economy are those bacteria which bring about the liberation of elemental nitrogen from nitrites and nitrates. These organisms may be called denitrifying bacteria. The denitrification process requires energy for the reduction of the nitrites and nitrates to elemental nitrogen. The required energy may be derived from the oxidation of carbon compounds, which is the energy source for most denitrifying bacteria, or the energy may come from the oxidation of sulphur. The latter process is carried on by *Thiobacillus denitrificans*. The denitrifying process takes place under anaërobic conditions. The oxygen of nitrite and nitrate is the source of oxygen for the respiration of the anaërobes. Those denitrifying organisms which oxidize carbon compounds in the soil are purely heterotrophs; the presence of nitrates

and organic matter favors their growth. The metabolic reactions may be represented as follows:

$$5C_6H_{12}O_6+24KNO_3 \rightarrow 24KHCO_3+6CO_2+18H_2O+12N_2$$

The decomposition of nitrogenous compounds free from nitrates either in the presence or absence of oxygen does not produce nitrogen gas. Under anaërobic conditions practically all of the nitrate nitrogen is liberated in gaseous form. Possibly the nitrate is reduced by nascent hydrogen produced in the anaërobic fermentation of glucose or organic acids, etc. The reactions may be represented as follows:



At high nitrate concentration and high temperatures, relatively large amounts of N_2O are formed, indicating the probability of these steps in the nitrate reduction.

The chemical reactions leading to nitrogen liberation may involve the interaction of nitrites produced by the bacteria with amino acids in the medium:

$$R\overset{\displaystyle H}{\overset{|}{C}}NH_2COOH+HNO_2 \rightarrow N_2+R\underset{\displaystyle OH}{\underset{|}{C}}H\ COOH+H_2O$$

There may be also the interaction of nitrite with ammonium salts to form ammonium nitrite which is relatively unstable and may undergo decomposition into water and nitrogen. $NH_4NO_2 \rightarrow 2H_2O+N_2$. These reactions might take place outside of the bacteria as well as inside.

The principal denitrifying bacteria are *Bacterium denitrificans*, an aërobe of importance in the liberation of the nitrogen of nitrates brought into the sea by river discharge: *Bacillus coli* and *Bacterium denitrificans agilis*, both of which are found in feces of cattle; various fluorescent bacteria, *Bacterium pyocyaneum* and *Bacterium hartlebii*. The organisms found in feces are of considerable importance in producing nitrogen losses from manures.

The removal of the nitrate radical from nitrates sets free the cation, which may lead to an alkaline reaction in the medium.

Thiobacillus denitrificans can oxidize thiosulphate only in the presence

Fig. 23. Jean Baptiste Joseph Dieudonné Boussingault, [illegible]

"The arrest of all further growth in the organism, after germination, when the seed deprived of fertilizer is formed of only an unweighable quantity of material, probably offers the most striking proof by which alone it is most easy to establish that the nitrogen which is in the gaseous state in the atmosphere is not directly assimilable by plants."

Agronomie, chimie agricole et physiologie. Ed. 2, Vol. 1, p. [illegible], [illegible]

of nitrates as a source of nitrogen. Its metabolic reactions may be represented as follows:

$$6KNO_3 + 5S + 2CaCO_3 \rightarrow 3K_2SO_4 \text{ [illegible] } 2CO_2 + 3N_2$$

Oxidation of carbon and sulphur compounds by the oxygen of nitrates is a relatively efficient process because the decomposition of nitrate to nitrogen does not require much energy.

6. NITRATE AND NITRITE REDUCTION

A relatively large number of bacteria, either obligate or facultative anaërobes, cause the reduction of nitrates to nitrites, and a number also produce ammonia from nitrites. The stage to which nitrate reduction proceeds depends probably upon the reduction potentials produced in media by the metabolism of the organisms and upon the oxidation potential required for their growth.

7. THE NITROGEN CYCLE

The beneficial effects resulting from the growth of legumes before cereal crops was observed by Theophrastus (about 372–287 B.C.) and

Fig. 24.—Hermann Hellriegel, 1831–1895, and Hermann Wilfarth, 1853–1904.

"In their requirements of nitrogen, the cereals are dependent entirely upon the assimilable nitrogen compounds present in the soil, and their development always stands in direct proportion to the available nitrogen of the soil.

"Besides the soil nitrogen, the legumes have available a second source, from which they are capable of filling their nitrogen requirements when the first source is insufficient.

"This second source is the free elemental nitrogen of the atmosphere.

"The legumes have not of themselves the ability to assimilate the free nitrogen of the air, but it is made available to them through symbiosis with living organisms of the soil.

"To make the free nitrogen available to the legumes for nutrition, the mere presence of any lower organisms in the soil is not sufficient, but it is necessary that certain species of the latter enter into symbiotic relationship with the former.

"The root nodules of the legumes are not merely to be considered as reserve stores of proteins, but stand in a causal relationship with the assimilation of free nitrogen."

Untersuchungen über die Stickstoffnahrung der Gramineen und Leguminosen. Beilageheft zu der Zeitschrift des Vereins f. d. Rübenzucker-Industrie d. D. A., pp. 203–204. 1888.

Virgil (70–19 B.C.). The beneficial effects of allowing the land to lie fallow was known to the Hebrews, and fallowing one year in seven was required by the Mosaic law. Boussingault (Fig. 23), in 1838, was the first to recognize that the beneficial effects of leguminous crops was due to their nitrogen fixation. Boussingault demonstrated that nitrogen is not fixed

by higher plants other than the symbiotic legumes. But the demonstration that it was the symbiotic bacteria living in the nodules that fixed nitrogen and allowed legumes to grow in soils deficient in nitrogen was not accomplished until 1886 by Hellriegel and Wilfarth (Fig. 24). The root nodule organism was isolated and described by Beijerinck in 1888.

Non-symbiotic nitrogen-fixing bacteria were isolated in 1893 by

Fig. 25. Sergius Winogradski, 1856

We are certain now that the assimilation of gaseous nitrogen by living matter can result from the symbiosis of legumes with microbes; we know also th[illegible] of lower organism inhabiting the [illegible], green algæ or microbes, can transform free nitrogen into fixed nitrogen, but up to the present we have not known any determined species which could with certainty be designated as endowed with that function."

Sur l'assimilation de l'azote gazeux de l'atmosphere par les microbes. Comptes rendus, 116, 1385, 1388,

"Schloesing and Müntz were correct in attributing nitrification to a specific organism, a nitrifying ferment, whose natural habitat is the soil; this microbe can be isolated and develops abundantly in appropriate solutions, in exercising the function which is proper to it."

Recherches sur les organismes de la nitrification. Annales de l'Institut Pasteur, 4: 213–231, 1890.

"Since this bound carbon in the cultures can have no other source than the CO_2 and since the process itself can have no other cause than the activity of the nitrifying organism, no other alternative was left but to ascribe to it the power of assimilating CO_2.

"Since the oxidation of NH_3 is the only source of chemical energy which the nitrifying organism can use, it was clear *a priori* that the yield in assimilation must correspond to the quantity of oxidized nitrogen. It turned out that an approximately constant ratio exists between the values of assimilated carbon and those of oxidized nitrogen."

Die Nitrification Handb. d. tech. Mykol. Vol. 3, p. 166ff. 1904–1906.

Winogradski (Fig. 25), who described *Closteridium*, and in 1901 by Beijerinck (Fig. 26), who named the genus *Azotobacter*. The work of these two men and their students is responsible for most of our information on the nitrogen, sulphur, and iron bacteria and their rôles in soil processes.

Pasteur suggested that the purification of sewage by the formation of nitrates is a bacterial process. The proof of this and the conditions of nitrification of sewage were demonstrated by Schloesing and Müntz in 1877.

The process of reforming nitrates from proteins in the soil is brought about by bacteria operating in turn. The production of ammonia from

Fig. 26.—Martinus Willem Beijerinck, 1851–

"As 'oligonitrophiles' I understand those microbes which in free competition with the rest of the microbe world develop in nutrient media, without intentionally added nitrogen compounds, but also without making provision to exclude the last traces of these compounds. They have the ability to fix the free atmospheric nitrogen and can use it for their nutrition.

"They give occasion to two principally different series of accumulation experiments. For instance, one can allow their development to take place: firstly, in light, at the expense of atmospheric carbon dioxide whereby the oligonitrophiles colored by chromophyl are to be expected; secondly, in presence of carbon containing nutrients in the dark, whereby colorless oligonitrophiles can be expected."

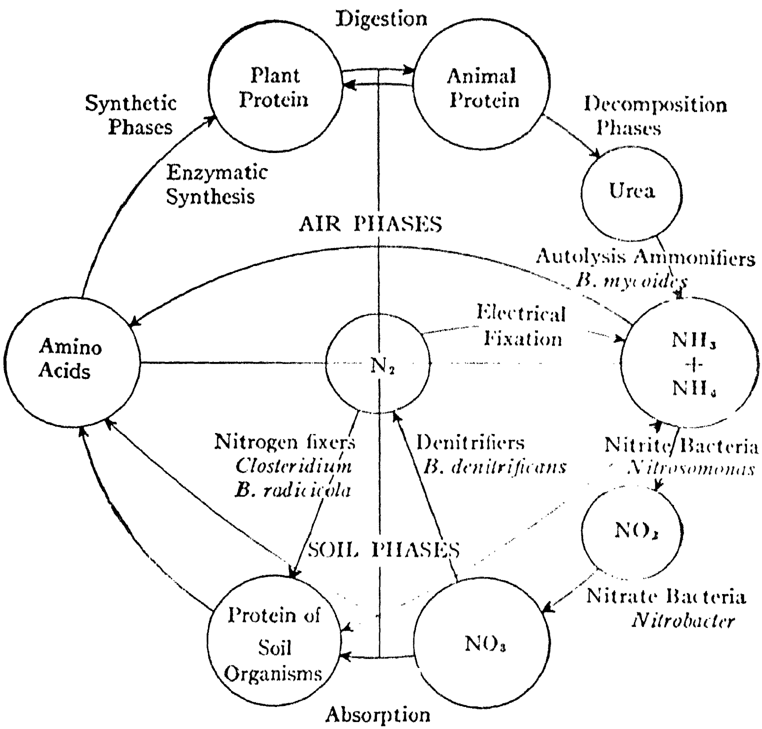
Ueber Oligonitrophilemikroben. Centralblatt für Bakteriologie, Parasitenkunde u. Infektionskrankheiten, II. 7: 561–582. 1901.

protein is brought about by the extensive ammonifying group of bacteria (*Bacillus mycoides*, etc.). This group of organisms is necessarily first in operation. They reduce the concentration of organic substances which in general inhibit the action of the organisms which are responsible for nitrification or the oxidation of the ammonia to nitrates. Evidently the ammonification process is generally not as quickly accomplished as nitrification. The production of ammonia is not extensive under natural conditions; free ammonia occurs mostly under conditions which prevent the action of nitrifying organisms. The transformation of ammonia to

nitrite by the nitrite-forming organisms (*Nitrosococcus*, *Nitrosomonas*) is evidently rapid. The oxidation of nitrite by the nitrate bacteria (*Nitrobacter*) is still more rapid, for nitrites seldom occur in soils. Nitrites may be found in sewage waters, but not in high concentrations. The actions of the bacteria occur in order, each organism is dependent upon its predecessor to form the products on which it can act. The stage to which nitrification can proceed is dependent upon the organisms present.

We may represent the cycle of nitrogen transformations as in the following diagram (Table 13).

TABLE 13



PART II

PART II

CARBOHYDRATES

CHAPTER IV

CLASSIFICATION AND PROPERTIES OF CARBOHYDRATES

I. *Importance of Carbohydrates as Plant Constituents*

The greater part of the dry matter of most plants is made up of carbohydrates. This group of substances makes up the framework of both herbaceous and woody plants. Especially in the latter the carbohydrates form structural elements giving mechanical strength to the plant parts. The carbohydrates represent the chief storage forms in plants and from them directly or indirectly nearly all of the organic compounds of the plant and animal kingdoms have been built up.. Necessarily nearly all of the energy for the organic world has come through the intermediate form of carbohydrates by the process of photosynthesis.

In the mature tree, carbohydrates make up many times as much storage material as any other substances. In animals the energy storage reserve is in the form of fats principally, with some storage as carbohydrate in the liver in the form of glycogen. The proteins make up a large part of the animal body. A comparison of the general chemical composition of the two kingdoms, plant and animal, can be had from the analysis of potatoes or sugar-beets, compared with analyses of animal carcasses. In plants the proteins represent a minor part. In woody plants the proteins may be found only in the outer living layers of wood and in the inner cortex. The proteins of the wood are gradually removed as the xylem cells grow older and as their protoplasm becomes more vacuolated. When the old xylem cells finally die, their protein constituents probably are autolyzed and move outward, being absorbed by the newly formed cells. It would then appear unnecessary for a tree to absorb each year from the soil all the nitrogen needed for new cell formation since part of this may come from proteins already present in old cells near the cambial layer. This process of removal of protein substance from old to new cells is of especial importance and interest in such trees as the eucalyptus and the sequoias, which grow to great height. In such plant structures it

appears that the proteins are comparatively quite evanescent, moving outward with the shell of growth. The carbohydrates of the wood, in comparison, are stable physiologically, and are not subject to removal or transport, but remain functional for centuries in their original position.

II. *Definition of Carbohydrates*

The term *carbohydrate*, as originally introduced, was made descriptive of the chemical elements which the group contains. The elements carbon, hydrogen, and oxygen were found to be present in carbohydrates in the proportion of two atoms of hydrogen to one of oxygen, the same ratio in which these are combined to form water, and for each atom of oxygen there is found one atom of carbon. So carbohydrates might be regarded as hydrated carbon. But this is not exactly pertinent to the group, for there are compounds showing greatly different properties from the carbohydrates which have a similar constitution. For instance, acetic acid and lactic acid have the three elements carbon, hydrogen, and oxygen present in the proportion given above, yet no one would classify them as carbohydrates. Also, there are substances which have the properties of carbohydrates which do not have the constitution demanded by this proportion. For example rhamnose, which has one hydrogen of the pentose sugar molecule replaced by a methyl group, making the ratio of hydrogen to oxygen other than 2:1.

Chemically, all carbohydrates are aldehyde or ketone derivatives of polyatomic alcohols, the molecules of which contain one carbonyl group $\overset{|}{\underset{|}{C}}=O$ and one or more hydroxyl groups —OH, one of the latter being attached to the carbon atom next joined to the carbonyl group. An example of this constitution is given in Fischer's formula for d glucose. Starting with the end of the chain to which the aldehyde or ketone group is attached, the carbon atoms are designated by the letters of the Greek alphabet as follows:

$$\begin{array}{cccccccccccc}
H & & OH & & H & & OH & & OH & & \\
| & & | & & | & & | & & | & & \\
C & — & C & — & C & — & C & — & C & — & CH_2OH \\
\| & & | & & | & & | & & | & & \\
O & & H & & OH & & H & & H & & \\
 & & \alpha & & \beta & & \gamma & & \delta & &
\end{array}$$

Carbohydrates then contain alcohol and carbonyl groups, and they show the reactions characteristic of these groups. Formaldehyde, according to the definition, is not a carbohydrate; it has no alcohol group

$$\begin{array}{c} \mathrm{H} \\ | \\ \mathrm{H{-}C{=}O.} \end{array}$$

Sometimes it is considered as the simplest sugar. Formaldehyde shows many properties which should properly separate it from the group of sugars.

III. *Optical Properties of Sugars*

From the formula for d-glucose it is seen that four carbon atoms, α, β, γ, and δ have attached to them four different groups. The α carbon bears the groups —CHO, —H, —O—H, and the chain of four carbons. These groups can be arranged in two orders: clockwise, or counter-clockwise, as follows: $4{-}\overset{1}{\underset{3}{\mathrm{C}}}{-}2$ or $2{-}\overset{1}{\underset{3}{\mathrm{C}}}{-}4$. These two arrangements cannot be made to coincide in space, and the carbon atoms are said to be asymmetric. If there are four asymmetric carbon atoms in the chain, then according to the law of permutations and combinations there are 16 or 2^4 ways in which the groups can be arranged in space. The arrangements of the groups in space determine the chemical properties of the substance, so that the 16 arrangements give 16 aldohexoses differing in their physical and chemical properties. For each arrangement or structural formula there is another which is the mirror image of it, which differs from it in the same way that one's right hand is different from the left hand. Such compounds, differing only in the spatial arrangement of their constituent groups, are called *stereo-isomers*. The asymmetrical arrangement of the groups causes the substances or their solutions to rotate the plane of polarized light either to the right, or clockwise direction, or to the left, or counter-clockwise direction, corresponding to the clockwise or counter-clockwise arrangement of the groups about the asymmetric carbon atom. Those substances which rotate the plane of polarized light to the right, or clockwise, are said to be dextrorotatory or d- forms; those which rotate the plane of polarized light to the left, or counter-clockwise, are said to be levorotatory or l- forms. The amount of rotation is specific for the substance, directly proportional to its concentration and to the length of the column through which the polarized light passes. If we know the length of the column and the concentration of the substance, we can determine a specific property, namely, the specific rotation (in angular degrees) of the substance. Conversely, if we know the specific rotation, the length of the column through which the polarized light passes, and the rotation in degrees, we can determine the concentration. The specific rotation of a substance is the rotation in angular degrees given by 1 gm. of a sub-

stance dissolved in 1 c. c. of solution when viewed through a tube 1 decimeter long. The specific rotation varies with temperature and with the wave-length of the light employed, so these conditions must be stated. The specific rotation is regularly taken at 20° C. with light of one wave-length only. The light emitted by a sodium flame is practically monochromatic, yielding only the D_1 and D_2 lines of the spectrum which are extremely close together, and this light is used in practice. Hence specific rotation is expressed as follows:

$$\text{specific rotation in degrees} \quad \left[\alpha\right]^{t}_{D_1} = \frac{\alpha}{l \times c}$$

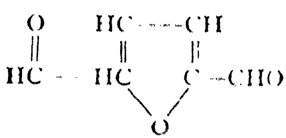
α is number of degrees rotation, t is temperature in degrees centigrade, l is the length of the tube in decimeters, c is the concentration in gram-molecules, D is the specification of the wave length of the light employed. The determination of concentration of sugar solutions and other substances by the polariscopic method is an extremely useful practice in commercial work.

IV. *Polymerization of Simple Sugars*

Aldehydes and ketones show a tendency to polymerize with the elimination of water, forming anhydrides. In similar manner complex carbohydrates seem to be derived from simple ones by the formation of anhydrides. Hydrolytic cleavage of these anhydrides again may yield the simple sugars.

V. *Chemical Test for Carbohydrates*

A general test for the carbohydrate group is the Molisch test, which is given by all carbohydrate groups whether pentose or hexose, and whether free or contained in combination in glucosides or in proteins. In carrying out the Molisch test take 5 c. c. of conc. H_2SO_4 in a test tube. Incline the tube to prevent mixing of the liquids, and slowly pour down the side of it about 5 c. c. of the sugar solution to which 2 drops of Molisch's reagent has been added. Molisch's reagent consists of a 15% solution of α-naphthol in alcohol which must be free from acetone. A reddish-violet zone is produced at the point of contact of the liquids, or a green ring with red above, changing on shaking to purple. The reaction is due to the formation of furfural from the carbohydrate by the acid.



VI. *Classes of Carbohydrates*

On the basis of their physical properties such as the property of crystallizing, the carbohydrates may be classified as follows:

1. Simple sugars—monosaccharides. Glucose and fructose are the only examples of this group which occur in quantity in plants in the uncombined condition. Most monosaccharides can be crystallized, but with some difficulty. This is probably because they exist in numerous isomeric forms in solution.
2. Complex crystalline sugars—disaccharides, trisaccharides, and tetrasaccharides. These sugars show no isomerism in solution and are easily crystallized.
3. Complex colloidal carbohydrates—polysaccharides, inulins, starch, plant gums, plant mucilages, celluloses, hemicelluloses. These substances generally exist in colloidal solution. Some may be crystallized by special procedures.

VII. *Classification of Simple Sugars*

Carbohydrates are classified according to the number of their carbon atoms into:

1. Monoses CH_2O.
2. Dioses $C_2H_4O_2$ CH_2OH CHO glycollic aldehyde.
3. Trioses $C_3H_6O_3$.
   a. Aldotrioses, l-glycerose CH_2OH CHOH CHO 2 isomers.
   b. Ketotrioses, dihydroxyacetone CH_2OH CO CH_2OH.
4. Tetroses $C_4H_8O_4$.
   a. Aldotetroses, 4 possible isomers, erythrose, threose.
   b. Erythrulose.
   c. Hydroxymethyltetrose, apiose.
5. Pentoses $C_5H_{10}O_5$.
   a. Aldopentoses, 8 isomers possible.
      Arabinose, xylose (ribose, lyxose).
   b. Methylpentoses, one of the hydrogen atoms of the primary alcohol group of aldopentose is replaced by a methyl group.
      Rhamnose found in glucosides.
      Fucose found in fucosan in *Fucus vesiculosis* and other brown algæ.
      Rhodeose in red algæ.
   c. Ketopentose, 4 possible, none found.
6. Hexoses.
   Aldohexoses, 16 possible, 14 known.
   a. Glucose series; mannose, glucose, idose, galose.
   b. Galactose series; galactose, talose, allose, altrose.
   c. Methylhexoses, artificially prepared, never found in plants.
   Ketohexoses, 9 possible isomers, 3 are known in plants. Fructose, sorbose, tagatose.
7. Heptoses $C_6H_{14}O_7$.
   Aldoheptose, glucoheptose, mannoheptose, galaheptose.
   Ketoheptoses. Sedoheptose from *Sedum spectabile*.
8. Octoses $C_8H_{16}O_8$.
   Aldo-octoses. Gluco-octose, manno-octose, gala-octose

9. Nonoses $C_9H_{10}O_9$.
   Aldononoses. Gluconononose, mannononose.
10. Decoses $C_{10}H_{20}O_{10}$.
   Glucodecose.

Ordinary glucose has a projection formula as follows:

CHO
|
HCOH
|
HOCH
|
HCOH
|
HCOH
|
CH_2OH

It may be represented as in the scheme of Willaman and Morrow by the symbol  and the same symbol may stand for all other compounds with different terminal groups and stereochemically similar to glucose, such as

COOH	CHO	
|	|	
HCOH	HCOH	CO
|	|	|
HOCH	HOCH	HCOH
|	|	|
HCOH	HCOH	HOCH
|	|	|
HCOH	HCOH	HCOH
|	|	|
CH_2OH	COOH	HCOH
		|
		CH_2OH
gluconic acid	glucuronic acid	

The dot signifies the aldehyde or ketone group. When the dot is removed, the symbol represents a compound having like terminal groups, as

$$
\text{for}\quad
\begin{array}{c}
\text{COOH}\\
|\\
\text{HCOH}\\
|\\
\text{HOCH}\\
|\\
\text{HCOH}\\
|\\
\text{HCOH}\\
|\\
\text{COOH}\\
\text{saccharic acid}
\end{array}
\quad\text{and}\quad
\begin{array}{c}
\text{CH}_2\text{OH}\\
|\\
\text{HCOH}\\
|\\
\text{HOCH}\\
|\\
\text{HCOH}\\
|\\
\text{HCOH}\\
|\\
\text{CH}_2\text{OH}\\
\text{sorbitol}
\end{array}
$$

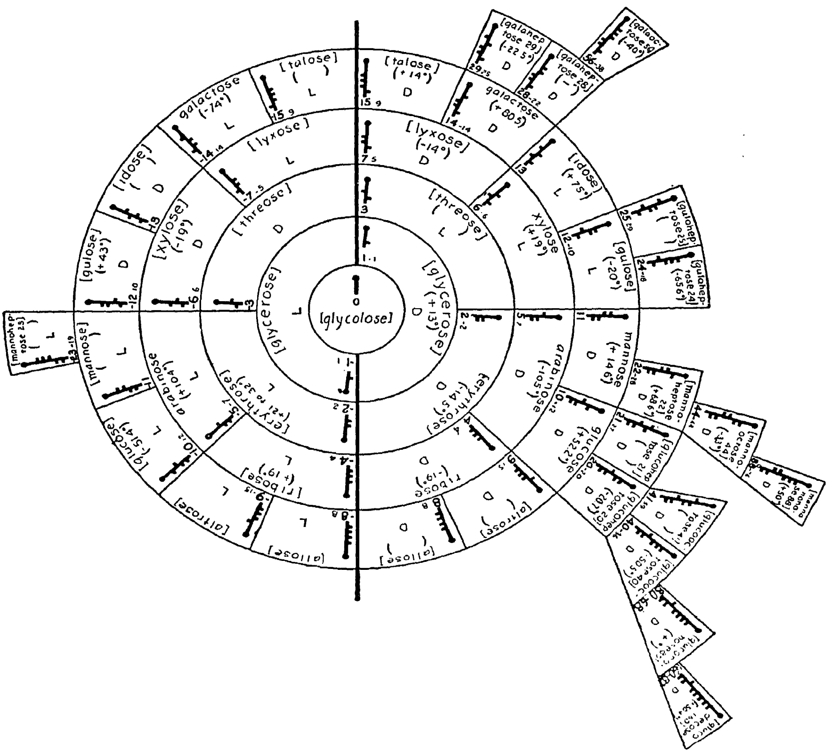


FIG. 27.—The structural relationships among the aldose sugars, together with data concerning their specific rotation, occurrence, and Fischer classification.

The flags represent the hydroxyl groups of the secondary alcohol groups, and thus also such groups as affect the stereochemical properties of the compound. In Fig. 27 the hollow circle represents the methyl group.

D-glycerose is represented as having its hydroxyl group to the right; l-glycerose, to the left. Hence, all the derivatives of d-glycerose fall in the right semicircle of the diagram, all have the basal hydroxyl group on the right, and all belong to the d- family of sugars.

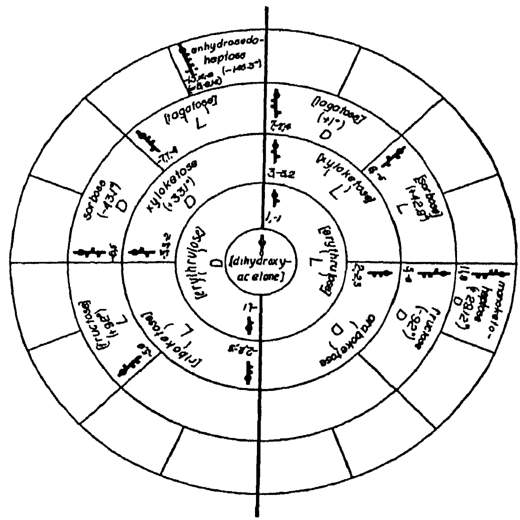


FIG. 28.—The structural relationships among the ketose sugars, together with data concerning their specific rotation, occurrence, and Fischer classification.

Figs. 27 and 28 show the structural relationships of the sugars. The index numbers under the symbols indicate the derivation of alcohols and dicarboxylic acids from the aldoses. Thus aldose 11 (d-mannose) leads to an acid and an alcohol not derivable from any other aldose. The designation 5_7 or 7_5 shows that two different aldoses, 5 and 7, the lyxose and arabinose of the same d- family, yield the same (active) acid and alcohol. The designation 14_{14} shows that the two enantiomorphous galactoses, 14 and 14, yield the same (hence optically inactive) acid and alcohol. Finally, the designation 10_{12} indicates that two aldoses belonging to the opposite families yield the same saccharic acid and sorbite; the aldoses not being enantiomorphous, the acid and alcohol must obviously be optically active. The antipodal acid and alcohol are derived from the aldoses -12 and 10 as indicated by the designation -12_{10}. The legends to the diagrams give the names both of the acid and of the alcohol derivatives of the aldoses, but only of the alcohol derivatives of the ketoses, and neither one in the case of the methyl aldoses.

The d's and l's indicate Fischer's classification. Four discrepancies occur in the case of the threoses, xyloses, guloses, and idoses. It is obvious that the individuals of these pairs, designated l by Fischer, belong to the right semicircle of their structure; and this is the basis for Rosanoff's

contention that the old designations for these four sugars should be reversed.

The family designations, d and l, show genetic relationships, and have nothing to do with the direction of rotation of polarized light.

Brackets around the name of a sugar show that it does not occur in nature. Thus d-glucose occurs naturally, but l-glucose does not; whereas both d- and l-gulose are synthetic, and both d- and l-arabinose are naturally occurring.

The specific rotation of the sugar is placed under the name in parentheses. Where no value is given, it indicates that the specific rotation is not known. When no parentheses are used, it indicates that the sugar has not yet been prepared. Thus d-allose has been prepared, but its specific rotation has not been determined; and l-allose is still unknown. Parentheses in the legend of the diagram indicate compounds not yet prepared.

It is usually considered that the members of any enantiomorphous pair have rotations which are equal, but opposite in character. Several exceptions to this appear in the diagram, as in the galactoses and erythroses. These discrepancies may be due (1) to inaccuracies in the determinations, (2) to impurity of the preparations, or (3) to a different point of equilibrium attained by the d- and l- forms of the two enantiomorphs. Whatever the cause, it is not justifiable to assume a value which has not been determined, as in the case of l-idose. Fischer named the aldoses having more than six carbon atoms after the hexoses from which they originated, as d-glucoheptose, d-manno-octose. In the case of epimers, Greek letters are in common use to designate the isomeric modifications of the mutarotating sugars, as α-d-glucose, β-d-glucose.

CHAPTER V

MONOSACCHARIDES

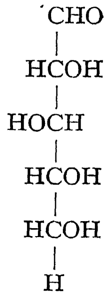
Dioses, trioses, and tetroses if existing at all in plants, are only transitory products, probably stages in synthesis or decomposition. If tetrose were formed it might easily go into some other substance which is more common, for instance, from the tetroses on mild oxidation the aldehyde group is changed to a carboxyl and active forms of the hydroxyacids are produced. On further oxidation, the primary alcohol group is also oxidized to carboxyl, yielding dibasic acids, *viz.:* d- and l-tartaric acids, and mesotartaric acid which is inactive.

COOH	COOH	COOH	COOH
|	|	|	|
HCOH	HCOH	HOCH	HOCH
|	|	|	|
HCOH	HOCH	HCOH	HOCH
|	|	|	|
COOH	COOH	COOH	COOH
a	b	c	d
meso-	d-	l-	meso

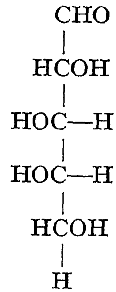
b and c are d- and l-tartaric acid, a and d are identical. They represent "internally compensated" mesotartaric acid. Tartaric acid is a common plant constituent, the four-carbon sugar is not. Octoses and nonoses are not found in plants. But they have been synthesized and some of their physiological properties are known. In plants pentoses and hexoses are the monosaccharides of importance and interest.

I. *Pentoses*

There are two pentoses common in plants, d-xylose and l-arabinose, and one methylpentose, rhamnose, which has one H of the pentose substituted by CH_3. l-ribose is not abundant, but is of importance as a constituent of nucleoproteins. The empirical formula for pentoses is $C_5H_{10}O_5$. The pentoses do not exist free in plants in any high concentration, but their polymers, the pentosans, are common. With proper hydrolytic enzymes, or when they are boiled with strong mineral acids, pentosans may be hydrolyzed to pentoses.



l-arabinose



All tissues giving a lignin reaction contain d-xylose. It is also found in bran, wood, straw, and the shells of apricot seeds, etc. l-arabinose is found in gum arabic and cherry gum. A pentose d-ribose forms part of the molecule of the nucleoproteins.

The pentosans xylan and araban $C_5H_8O_4$ ($C_5H_{10}O_5$—H_2O) are polymers of the pentoses. They are found in the skeletal structures of plants almost entirely. The enzymes that act upon them are named according to the particular pentosan upon which they act. For example, xylanase acts upon xylan.

Grüss found that the inner part of wood vessels was digested in spring with the formation of a gum. An enzyme was probably the active agent. The enzymatic hydrolysis of pentosans is not extensive in plants, but a great number of wood-rotting fungi, *Xylaria*, etc., regularly use these substances as a source of energy.

The gums contain pentosans in combination with complex organic acids. Gum arabic, according to O'Sullivan and Robinson, consists of 2-araban ($C_{10}H_{10}O_8$), 4-galactan ($C_{12}H_{12}O_{10}$) arabic acid ($C_{23}H_{30}O_{18}$). Gum of Gedda from one of the acacias consists of 4-araban, 3-galactan geddic acid. Gums, then, are not entirely carbohydrate. They contain a variety of complex organic acids combined with carbohydrate groups.

The pentosans can serve as reserve material when the more readily utilizable carbohydrates have been exhausted. In leaves the pentosans increase during the day and decrease at night. They increase when the leaves are supplied with glucose, and decrease when the action of the chlorophyl is prevented and carbohydrate nutriment is absent. Under illumination there is a high O_2 content in the intercellular spaces of the leaf, and this with high glucose content may account for the formation of pentoses and pentosans under this condition. Pentosans may be produced in stems under tension and compression. Dehydration of tissues also causes their production.

The methylpentose, rhamnose, is found as a constituent of many glucosides. The most widely distributed glucosides which contain rhamnose are flavone derivatives such as quercitrin, the red pigment of oak.

II. *General Properties of the Pentoses*

The aldopentoses have three asymmetric carbons; hence there are 2^3 or 8 stereoisomers. The pentoses are not fermentable by yeast; they are, however, usable by a number of fungi. On distillation with concentrated HCl or H_2SO_4 (25%) they are converted into furfural, which may be detected by its turning aniline acetate paper red.

$$C_5H_{10}O_5 - 3H_2O \rightarrow \underset{\text{furfural}}{C_5H_4O_2}$$

This reaction may be seen by boiling shavings or bran with concentrated HCl in a test-tube and allowing the steam to come in contact with filter-paper moistened with aniline acetate. A pink color is produced if pentosans are present. Hexoses also produce this reaction but to much less extent, giving about 2% as much furfural as the pentoses.

When warmed with concentrated HCl (sp. gr. 1.2) and a little orcinol, the pentoses produce a greenish-yellow compound which is soluble in amyl alcohol to a clear green solution with characteristic absorption bands between the C and D lines of the spectrum. This color reaction may be modified by adding a couple of drops of ferric chloride to the solution after it has been heated with HCl and orcin, producing a bright-green color. This test is characteristic for pentoses. The pentoses give the Molisch test, and they form osazones. Arabinose osazone melts at 157° C. Xylose osazone melts at 160° C. The pentoses reduce Fehling's solution because they have a free aldehyde group. No pentoses of the keto type are known in plants.

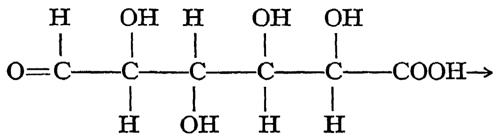
III. *Synthesis of Pentoses*

If the CH_2O groups were added successively on the condensation of formaldehyde produced in photosynthesis, it would be possible to get successively 2, 3, 4, 5, or 6 or more molecules condensed. A six-carbon sugar, acrose, has been synthesized in this manner. This would account for the origin of pentose in photosynthesis, but this method of origin of pentoses probably does not occur. If the sugars are formed from condensation of two molecules of glyceric aldehyde or other triose, only hexoses could be formed.

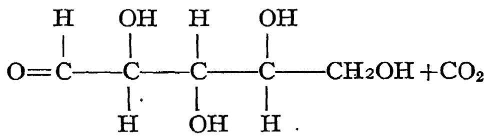
$$2\underset{\text{glyceric aldehyde}}{CH_2OHCHOH\ CH{:}O} \rightarrow \underset{\text{hexose}}{CH_2OH(CHOH)_4CHO}$$

This idea has been advanced, but there is insufficient evidence to establish it clearly. However, it seems fairly clear that pentoses are formed only from hexoses by oxidation.

In the aldose sugars the carbon of the carbonyl group is most reactive and is the cause of the great reactivity of these sugars. In di- and polysaccharides these groups are so united with other groups that this carbon is no longer reactive. The di- and polysaccharides are then attacked by oxidation at the opposite end, at the primary alcohol group, —CH_2OH. Such a reaction gives glucuronic acid $CHO(CHOH)_4COOH$. The presence of glucuronic acid in plants has been established. A general property of acids of the formula of glucuronic acid is to split off CO_2 from the carbonyl group in sunlight.



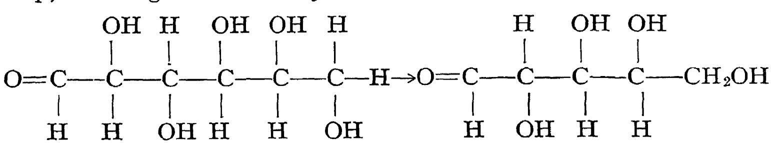
glucuronic acid



l-xylose

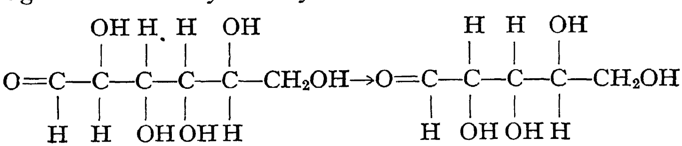
This reaction is commonly shown by bacteria which form xylose as a product of metabolism. It probably is concerned in the gummosis of trees.

If the pentoses were made from hexoses by oxidation of the aldehyde group, then d-glucose would yield d-arabinose.



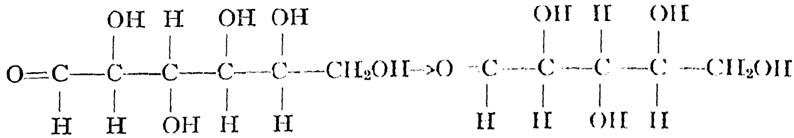
d-glucose d-arabinose

Also d-galactose would yield l-xylose.

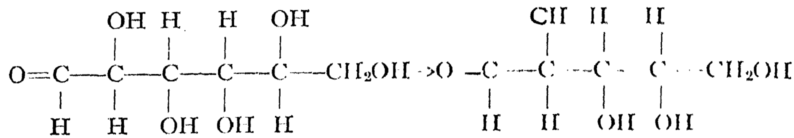


d-galactose l-xylose

But in plants the following are the sugars which are found associated: d-glucose with d-xylose, and d-galactose with l-arabinose.



d-glucose d-xylose



d-galactose l-arabinose

This association in plants gives evidence that glucuronic and galacturonic acids are intermediate compounds in the formation of pentoses from hexoses.

Pentoses on oxidation at the aldehyde end yield pentonic acids, for example, arabonic acid and xylonic acid. Pentonic acids on oxidation yield a tetrose, CO_2 and H_2O. But in plants this mechanism for the formation of tetroses does not seem to function. The primary alcohol group of the pentoses can be oxidized, yielding a dicarboxylic acid. Both xylose and arabinose yield trihydroxyglutaric acid, $COOH\ (CHOH)_3\ COOH$. On reduction, arabinose and xylose yield the corresponding alcohols, arabitol and xylitol.

The pentose polysaccharides have a marked property of taking on water in contrast to many of the hexosans. A high degree of hydration of the pentosans when in contact with water is an indication of the unsaturation of accessory valencies which allow the molecules to form loose compounds with water. This is not shown by the hexose polysaccharides such as starch or cellulose. Evidently then the nature of the linkages between atoms in pentosans and hexosans is somewhat different. Pentosans in the cell increase enormously the hydration capacity of the tissue. They are important in the water-holding power of cacti and other such plants. They enable plants to hold their moisture against drought or extremely low temperatures. The presence of pentosans favors the undercooling of tissues and retards ice formation.

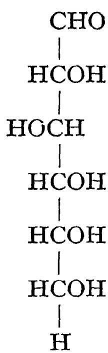
The pentoses originate in plants under conditions in which metabolic activity is repressed, such as in drought conditions or low temperature exposure, or on wounding, or when the tissue is under tension or pressure. There is a rather remarkable case of the formation of woody elements

containing pentoses when tendrils are subjected to tension. Pentoses may be regarded as important in the economy of succulents in arid regions since their formation results in increased water-holding capacity.

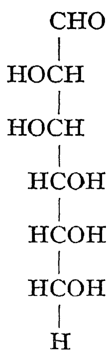
Pentoses can be differentiated from hexoses by boiling them with dilute 10% HCl or H_2SO_4, because they yield furfuraldehyde. The hexoses yield furfuraldehyde only with strong acids.

IV. *Hexoses*

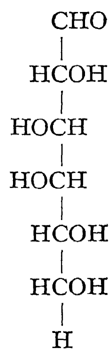
There are three aldohexoses common in plants, d-glucose, d-mannose, and d-galactose.



d-glucose

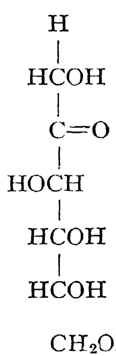


d-mannose



d-galactose

There is one ketohexose common in plants, d-fructose, and one rarely found, d-sorbose.



d-fructose or levulose

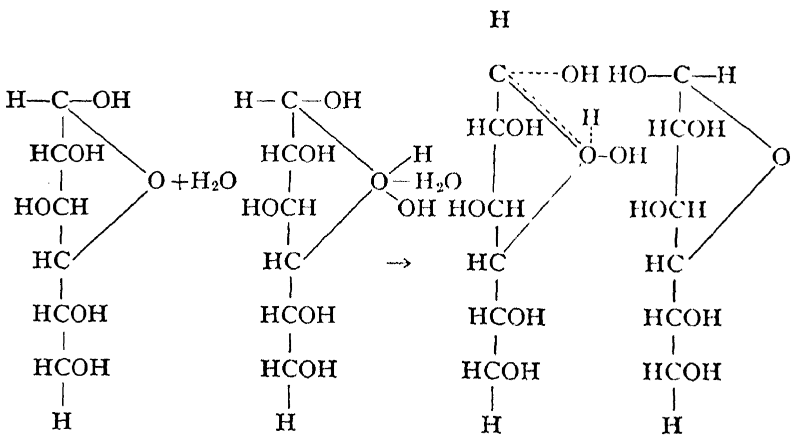
With this formula there are sixteen possible aldohexoses according to Van't Hoff's rule (2^4). Two closed-chain amylene oxide forms, two butylene oxide forms, two propylene oxide forms, and two closed-chain ethylene oxide forms are possible, corresponding to each of the open-chain aldehyde forms.

The hexoses are optically active.

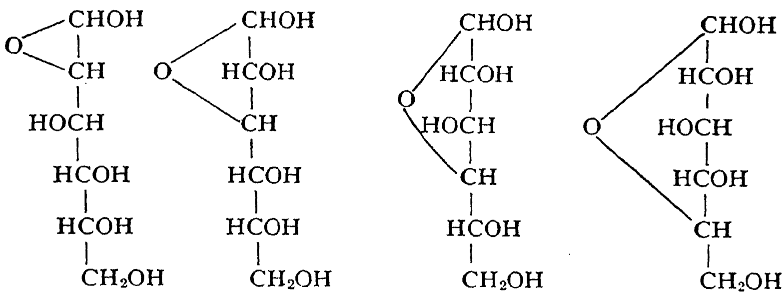
d-glucose $+52.5°$	d-galactose $+81°$
d-mannose $+14°$	d-fructose $-92°$

The specific angular rotation of d-glucose when first dissolved is $+110°$. This gradually falls to $+52.5°$. By the addition of a trace of alkali the rotation changes very rapidly to the lower value. This change of rotation indicates that there are two forms of d-glucose (α and β) differing in rotating power, one being formed from the other in water solution. This change is shown also by all the other d- and l-hexoses. There is evidence that the sugars in water solution form lactone ring compounds with the aldehyde oxygen atom in the lactone ring.

In α-d-glucose the oxygen of the butylene oxide ring is on the same side as the OH of the terminal aldehyde group. In β-d-glucose it is on the opposite side. The transformation on solution in water may be represented as follows:



α-d-glucose $[\alpha]_d = 113.4°$ — oxonium hydrate — OH can wander back to either α or β position — β-d-glucose $[\alpha]_d = +19°$



ethylene oxide form | propylene oxide form | butylene oxide form | amylene oxide form

β-d-glucose

This property of mutarotation or change of position of the H and OH groups is explained by Armstrong as due to labile forms existing in equilibrium.

D-glucose then may exist in the following forms: One form of the regular aldehyde formula, or Fischer formula; two forms, α and β, with the amylene oxide formula; two forms, α and β, with the butylene oxide formula; two forms, α and β, with the propylene oxide formula; and two forms, α and β, with the ethylene oxide formula. Gamma glucose is a mixture of α and β forms. At equilibrium in 10% glucose solution at 22° C. there is 37% α to 63% β. The presence of ions, the temperature, and the concentration of glucose alter the position of this equilibrium.

α-d-glucose crystallizes from solution at ordinary temperatures; β-d-glucose crystallizes out at temperatures above 98° C. The α and β forms are in equilibrium at these temperatures, but separation of one form as a solid phase causes reëstablishment of equilibrium at the expense of the other, so that it disappears and only one form crystallizes out.

The fact that glucose exists in the equilibrated α and β forms in solution is of great importance biologically. If the β form is more reactive or more easily metabolized, any condition driving the equilibrium in the direction of the β form will increase the rate of reaction or metabolic change. All natural occurring glucosides are of β-d-glucose. This indicates a higher reactivity of the β form.

If CH_3 is joined to the H of the OH in the α-d-glucose terminal carbon, the ring is so stabilized in the α-methyl glucoside resulting, that it does not reduce Fehling's solution or react with phenylhydrazine because it does not undergo hydrolysis as the α-d-glucose does to form the straight chain aldehyde group. The β form of methyl glucoside is more easily

attacked than the α form, again indicating greater reactivity of the β form. α-methyl glucoside is hydrolyzed by maltase. β-methyl glucoside is hydrolyzed by emulsin. These enzymes are specific and more active than acids as hydrolytic agents of the glucosides. Yeast contains maltase and will act on the α-glucoside converting it to methyl alcohol and α-d-glucose which may be fermented, leaving pure β-methyl glucoside from a mixture of α- and β-methyl glucosides.

In the plant, glucose may occur as the uncombined substance which may be crystallized within the tissue on desiccation. The onion, the grape, and numerous other plants contain free glucose. It should be remembered that this glucose may exist in several different configurations which may differ in their reactivity. Probably all possible forms of α glucose occur in the plant. The condensation of the different isomeric forms of glucose may lead to the formation of different anhydrides. The anhydrides of glucose occur extensively in plants, forming cellulose, starch, and dextrins. Glucose also is a constituent of polymers which contain other hexoses. Sucrose containing a molecule each of glucose and fructose is a very common constituent found almost universally in plants.

Fructose occurs in most all plants as the free hexose, or combined to form sucrose. In certain families of plants, particularly the grasses and the COMPOSITÆ, the anhydrides of fructose form important reserve foods. In dahlia tubers and in artichokes the anhydride of fructose, inulin, is of as great importance as the starches of other plants. In the grasses similar anhydrides, for instance phlein in *Phleum pratense* (timothy), are found. There are numerous fructosides and inulides occurring in plants.

Mannose occurs mostly in the form of its anhydrides, the mannans. These substances are commonly deposited as secondary thickenings in the cell wall of the endosperm of the date (*Phœnix dactylifera*) and in palm seeds. Mannose is produced upon the hydrolysis of these reserve substances when they are digested during the germination of the seed. Mannose occurs also as a constituent of some gums and mucilages.

Galactose occurs in plants mostly as the anhydride, galactan, in the cell walls of woody tissues. It is a constituent also of gums and mucilages.

Monosaccharides in aqueous solution are relatively stable, but in living protoplasm they are unstable and undergo transformations easily. There is no difficulty in transforming one form into another in the protoplasm, although the change may be difficult for the organic chemist.

V. *Ionization of Sugars and Their Transformations*

The sugars which contain several OH groups act as very weak acids. They form salts with metals as in the case of calcium sucrate. The dissociation of the sugars as weak acids is very low, being as follows:

Glucose	6.6×10^{-13}
Fructose	9.0×10^{-13}
Saccharose	2.4×10^{-13}
Maltose	18.0×10^{-13}
Mannose	1.09×10^{-12}
Galactose	5.2×10^{-13}
Raffinose	1.8×10^{-13}
Lactose	$6. \times 10^{-13}$

pared with the ionization of some oth

Dulcitol	3.5×10^{-14}
Mannitol	3.4×10^{-14}
Glycerin	7×10^{-15}
Ethyl alcohol	10^{-15}
CO_2	3.04×10^{-7}
H_2O	$1 \times 10^{-7.07}$
Tartaric acid	1×10^{-3}
Acetic acid	1.8×10^{-5}
Lactic acid	1.5×10^{-5}
Butyric acid	1.5×10^{-5}

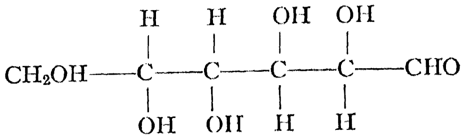
This ionization, although small, is the primary condition leading to the transformation which sugars undergo. The reactions of organisms take place in the presence of numerous ions which affect the ionization of the sugars markedly. Owing to their weak ionization the sugar ions are easily decomposed by such weak acids as CO_2. Sucrose is almost completely hydrolyzed in ten minutes at 100° C. in solutions whose acidity is due to CO_2 alone.

The metals unite with the sugars, forming an R—CH—O—M group. The salts so formed from sugars are more highly ionized. Iron accelerates markedly the oxidation of glucose in solution by the air and Fehling's solution increases the sugar ionization. In pure water glucose remains as α and β forms for years unchanged.

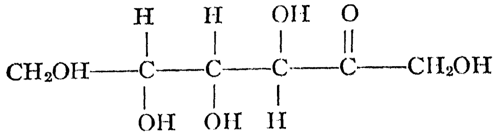
In weak alkalies the groups in the sugar molecule undergo transformation, so that a variety of substances may be formed from one molecular arrangement, the different forms existing in equilibrium. Starting with either d-glucose, d-mannose, or d-fructose with 1/20 n $Ca(OH)_2$ there are established the same equilibrium substances in each case. There is produced a mixture of the following as represented by the Fischer formulæ:

$$\begin{array}{ccccccccccc} & & H & & H & & OH & & H & & \\ & & | & & | & & | & & | & & \\ CH_2OH & — & C & — & C & — & C & — & C & — & CHO \\ & & | & & | & & | & & | & & \\ & & OH & & OH & & H & & OH & & \end{array}$$

d-glucose



d-mannose

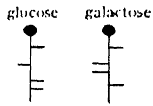


d-fructose

The changes involved are merely shifting the position of the H and OH group, and the substances produced are said to be *epimerides.* The change in position of H and OH is called *epimerism.*

In a similar manner, with dilute alkali, d-galactose yields a mixture of d-galactose, d-talose, d-tagatose, and d-sorbose.

A member of the d-glucose series is never transformed into a member of the d-galactose series. The difference between d-glucose and d-galactose lies in the space relation of the OH on the γ or third carbon atom from the carbonyl group.



It is evident that the enolization changes involve only the three carbons next to the aldehyde CHO group. There exists a gradient of reactivity in the carbons beginning at the carbon atom adjacent to the aldehyde CHO group, and decreasing in reactivity the farther away from the aldehyde CHO group one proceeds, until the γ carbon atom is reached when the reactivity has fallen almost to zero.

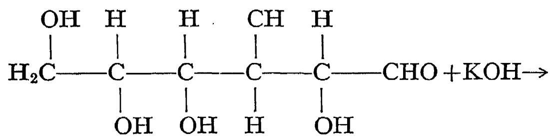
D-glucose and d-fructose are by far the most abundant sugars found in plants. According to the equilibrium concentrations of the various sugars formed by enolization in the glucose series, the aldoses and ketoses are about equal in amount. This would indicate a cause for the appearance under natural conditions in plants of one molecule of glucose for each molecule of fructose, as in sucrose. Of the aldose sugars in the glucose series, d-glucose represents five times as much as d-mannose, and this is similar to the proportions of these sugars found in plants. In the galactose series at the equilibrium point, d-galactose represents nine times as much as all other sugars of this group, and this relation holds fairly well for the importance of these sugars as constituents of plants. In enolization, no

d-allose or d-altrose was formed from d-glucose, and no l-gulose or l-idose was formed from d-galactose. These sugars also do not appear in plants.

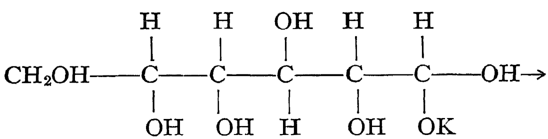
Evidently the equilibrium concentrations of the various sugars produced by enolization closely approach the conditions existing in nature. The pH reaction of protoplasm is proper in many cases for such transformations to be brought about. Also, there are probably catalysts in the cell which may bring about the transformations.

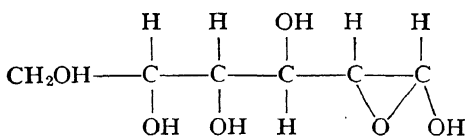
In plants such as the Compositæ, where the common storage form is fructose anhydrides, some condition evidently favors the formation of this isomer instead of the glucose anhydride found in the greater number of plant families. Enzymes do not change the position of the equilibrium between forms, but change the rate of establishment of the equilibrium, or may initiate the transformation.

In strong alkaline solutions further enolization of the sugars occurs:

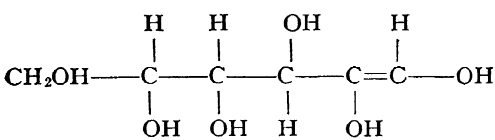


d-glucose





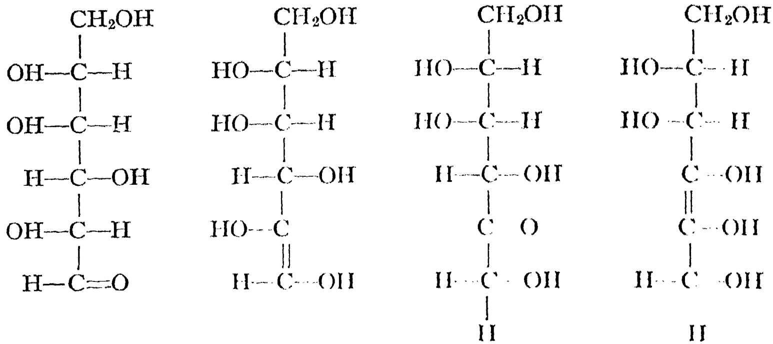
ethylene oxide form



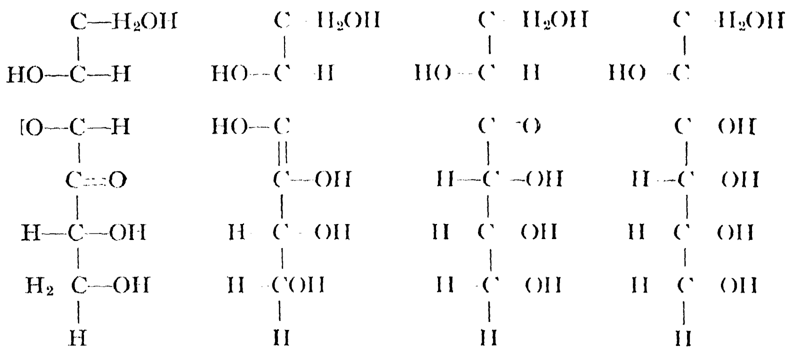
1-2 dienol form of d glucose, d-mannose, or d-fructose

By taking up one molecule of H_2O at the double bond and rearrangement this enol goes over into d-glucose, d-mannose, or d-fructose, accord-

ing to whether the OH of the HOH attaches to carbon 1 or 2. In this manner the hexoses are interconvertible. In the same way there can be formed:



d glucose 1 2 dienol fructose 2 3 (

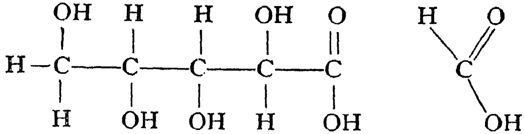


3 4 dienol 2 3 dienol fructose

These forms are also capable of rearrangement to form all possible arrangements in the saccharide molecule.

A general property of double bonds in carbon compounds is their high reactivity as shown in fatty esters of the unsaturated fatty acids, etc. When iodine or bromine is introduced into the chain, the double bond opens easily to combine with the new groups. Similarly, the enol forms of the sugars are very reactive and break apart spontaneously at the double bonds with a result that highly reactive fractions of the sugar molecule are formed. The shifting of the position of the double bond on enolization by alkalies would account for the breaking of a hexose into 5 1, 4 2, or 3 3

carbon atom pieces. Breaking of the 1–2 dienol results in formation of formic and d-arabonic acid, if oxygen is present.



The ketose sugars are decomposed in aqueous solution by ultra-violet light. Carbon monoxide is evolved and the corresponding alcohol containing one carbon atom less is formed. The aldo sugars are practically unaffected by these conditions. Evidently this difference in stability is due to difference in the molecular arrangement.

VI. *Determination of Reducing Sugars*

Among the important chemical characters of monosaccharides which apply to both hexoses and pentoses is that of showing the power of reducing various substances. They reduce an ammoniacal silver solution forming a mirror of silver; they form resin-like substances on treatment with alkalies, the resins being complex polymers. The reduction of Fehling's alkaline cupric tartrate solution is a reaction of importance in qualitative and quantitative work on the sugars.

Fehling's solution is made from a mixture of two solutions: Solution, I, $CuSO_4 \cdot 5H_2O$ solution containing 69,278 gms. per litre; and Solution II, alkaline sodium and potassium tartrate solution containing 348 gms. sodium and potassium tartrate and 100 gms. NaOH per liter. The solutions are made up separately and mixed immediately before use to prevent reduction in the mixture itself. The purpose of the alkali and tartrates is to increase the ionization of the sugars and to produce the enol forms. This increases the reactivity of the sugar molecule so that it is more easily oxidized. The hydrogen-ion concentration is also established at a proper value by the mixture. The oxidation of sugars proceeds most rapidly in alkaline media.

When a sugar is added to this mixture, if it contains reactive free aldehyde or ketone groups it will be oxidized through a great variety of substances of 1, 2, 3, 4, 5 carbon-atom chains with the simultaneous reduction, of the cupric ion to cuprous ion which separates as Cu_2O. Complex copper compounds are produced as intermediates in the reduction, and these dissociate readily. As the cupric ions are used up by reduction, more are supplied from the tartrate complex. The cupric ions must always be kept in excess to get quantitative reduction. Various reducing sugars differ greatly in their reducing power under standard conditions.

The conditions under which the Fehling's reaction is carried out are important. The time required to come to the boiling-point and the length of the boiling period must be accurately adjusted to get quantitative results. Once the reduction is accomplished, several methods may be used to estimate the cuprous oxide produced. Volumetric titration methods are convenient when there are no interfering substances in the solution. In the gravimetric method the weight of Cu_2O may be determined or it may be redissolved and the copper determined electrolytically. These methods do not distinguish between the various monosaccharides or other reducing substances which may be present in plant tissues.

The reduction of Fehling's solution is possible only where there is a free aldehyde or ketone ending. Some disaccharides, maltose, and lactose reduce Fehling's solution because they have free aldehyde or ketone groups. Tannins also reduce Fehling's solution.

VII. *Formation of Hydrazones and Osazones*

With phenylhydrazine and substituted phenylhydrazines the sugars first yield hydrazones with the elimination of H_2O, and then on further warming in an acetic acid medium osazones are produced. The reaction is through a $\overset{|}{C}=O$ (keto sugars) or $H—C=O$ group (aldo sugars).

$$\underset{\text{glucose}}{CH_2OH(CHOH)_4\,CHO} + \underset{\text{phenylhydrazine}}{H_2}$$

$$\underset{\text{glucose phenylhydrazone}}{CH_2OH(CHOH)_4CH=N-NHC_6H_5} + H_2N-N\cdot-C_6H_5 \rightarrow$$

$$\begin{array}{llll} CH_2OH(CHOH)_3 — & C — & CH & NNHC_6H_5 \\ & \| & \| & \\ & N — & N — & C_6H_5 \end{array}$$

$$\text{glucosazone}$$

Excess of phenylhydrazine oxidizes the next CHOH to the CHO which again reacts to introduce a second group. Both carbons at the aldehyde or ketone end are acted upon; hence the same osazone is produced by d-glucose, d-fructose, and d-mannose. Glucosamine derived from chitin also gives the same osazone as these sugars.

Methylphenylhydrazine gives a reaction with the keto group of d-fructose, and then the action stops without production of an osazone. The aldoses do not react with methylphenylhydrazine. Hence this is a reagent for differentiating between keto and aldo sugars. Ketoses are more reactive sugars than the aldoses.

The osazones dissolve in water with difficulty, a property of service

in the separation of the monosaccharides which are all very soluble in water and may crystallize with difficulty, especially in the presence of salts. Mannose is different from the other hexoses in that it forms in neutral medium an insoluble phenylhydrazone by which it may be identified. The osazone does not form so easily because the OH is on the other side of the chain from its position in glucose. The different osazone crystals are rather characteristic, and the sugars can be more or less readily identified from their osazones. The melting-point of the osazones is different and may be used for identification. Phenylhydrazine is much used in microchemical tests for sugars. Rosing used this method to determine sugars in the guard cells at different times of the day. With practice one can learn to distinguish the hydrazones and osazones of different sugars in plant tissues. It must be remembered that the crystal form will be modified by the presence of sugars and other substances in the plant.

VIII. *Oxidation of Hexoses*

The main source of energy in plants is that liberated from the oxidation of sugars in respiration. The oxidation of glucose by weak oxidizing agents begins at the aldehyde end, forming an acid, gluconic acid.

CHO	COOH
|	|
HC—OH	HCOH
|	|

The gluconic acid may be precipitated as the calcium salt and identified.

Further oxidation of glucose (*e.g.*, with dilute HNO_3) attacks both ends of the carbon chain, and saccharic acid is the result. The monopotassium salt of saccharic acid is insoluble and may be used in its separation. Other aldoses yield similar products. The changes on oxidation may be represented as follows:

CHO	CHO	COOH	COOH
HCOH	HCOH	HCOH	HCOH
|	|	|	|
HOCH	HOCH	HOCH	HOCH
|	|	|	|
HCOH	HCOH	HCOH	HCOH
|	|	|	|
HCOH	HCOH	HCOH	HCOH
|	|	|	|
CH_2OH	COOH	CH_2OH	COOH
glucose	glucuronic	gluconic	saccharic

Similarly there may be formed:

mannose	mannuronic	mannonic	mannosaccharic
galactose	galacturonic	galactonic	mucic

Galacturonic acid is found in lemons and in the pectin of sugar-beets. A great many pectic substances have been found to be derivatives of galacturonic acid. This acid must be regarded as playing an important part in the structure of plant cell-wall materials.

Galactose when oxidized with dilute HNO_3 yields mucic acid which is sparingly soluble and is used as a means of identifying galactose. About 5% of cherry gum produces mucic acid on oxidation, owing to the presence of galactans. Galactose occurs commonly in galactosans, and also as galactosides as in the saponins. Galactose is abundant enough to form a white coating over *Parthenocissus* berries after they are frozen. Galactozymase is required for the fermentation of galactose, since ordinary zymase does not attack this configuration of the hexose.

Under abnormal conditions galactose is formed in the sugar-beet, and it appears in combination with sucrose as the trisaccharide raffinose. The quantity of raffinose is increased by disturbances of growth such as those occasioned by sudden frost. Under this condition the galactans probably are hydrolyzed and yield galactose. The free galactose is combined first with glucose to form the disaccharide melibiose. Then the glucose half in this disaccharide according to fixed habit is combined with fructose to form raffinose.

The ketohexoses in oxidation break at the ketonic group, yielding two acids with various numbers of carbon atoms, depending upon the position of the ketonic group in the chain.

IX. *Reduction of Hexoses*

On reduction d-glucose takes up two H's at the aldehyde end, forming a hexahydric alcohol, sorbitol.

$$\begin{array}{c} OH \\ | \\ H—C—H \\ | \\ HCOH \end{array}$$

Mannose on reduction gives mannitol, galactose gives dulcitol, fructose gives a mixture of sorbitol and mannitol. The pentoses on reduction give their corresponding alcohols, xylose gives xylitol, arabinose gives

arabitol. All these alcohols formed from monosaccharides are found in plants. Mannitol, for instance, is found in mushrooms that have been lying some time around the market. It may exceed glucose in quantity, replacing it as a storage form. Mannitol is used as a source of energy by many fungi and bacteria. Dulcitol is more resistant to oxidation on account of its configuration. The flavors of mushrooms, celery, and asparagus are due to these alcohols in part. Sorbitol is a common constituent of *Sorbus* berries.

With regard to the method of formation of these reduced products there is a possibility that they may originate when the oxygen of glucose is used in certain cases by fungi as a substitute for atmospheric oxygen. Glucose is generally a reducer in respiratory processes in the higher plants, *i.e.*, it is oxidized itself. Evidently only a small percentage of the energy of glucose becomes available when the oxygen for the formation of CO_2 comes from a part of the glucose molecule itself. Strongly reducing conditions may lead to the formation of these alcohols. It has been found that they may be produced from hexoses, or pentoses, by reduction with gaseous hydrogen in the presence of platinum as a catalyst.

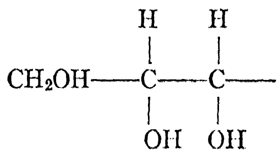
CHAPTER VI

USE OF SUGARS IN METABOLISM

I. *Relation of Isomerism to the Use of Sugars*

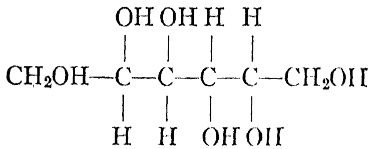
There is a high specificity of organisms in the use of sugars and related compounds.

Bacterium xylinum can oxidize only compounds having the grouping

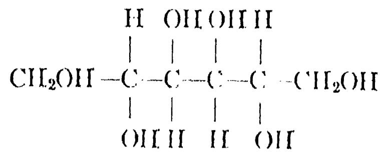


It can oxidize mannitol but not dulcitol.

Consideration of the formulæ of mannitol and dulcitol will help to make this clear:

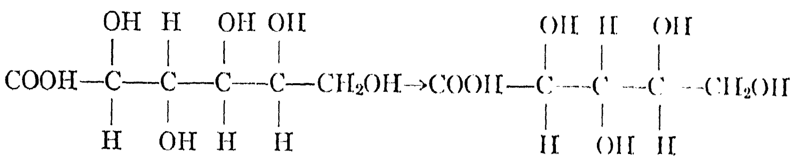


mannitol—converted into fructose



dulcitol—not attacked

Gluconic acid contains the grouping required by *B. xylinum*. Accordingly, it is further oxidized by the bacterium to ketogluconic acid:



gluconic acid ketogluconic acid

In contrast with the sucroclastic enzymes, which are apparently in harmony with the sugar molecule as a whole, these oxidizing bacteria seem adapted to a section only of the molecule. Their action is none the less absolutely dependent on the presence of the requisite configuration

in the molecule. We may explain this by saying that the enzymes have a definite structure or catalytic power corresponding to the groupings which are acted upon.

Many bacteria which are without action on dulcitol act upon mannitol. Harden found this to be true for *Bacillus coli communis*, which is of interest also since it produces twice as much alcohol from mannitol as from glucose. This difference is ascribed to the presence of the group $CH_2OH—CHOH$ which is contained only once in glucose but twice in mannitol.

Only those bacteria which produce fermentation of glucose act on pyruvic acid, $CH_3—CO\ COOH$.

The fermentation of various carbohydrates and allied substances by bacteria is effected by a single set of enzymes the action of which is common to all such cases of fermentation. The first step in the alteration of a particular molecular structure may require a special enzyme to produce the common intermediate substance, but the subsequent changes are always similar, being due to the action of the standard series of bacterial enzymes.

A further example of the influence of configuration on physiological properties is afforded by the formation of the urease ferment by bacteria. While d-glucose, d-galactose, and d- and l-arabinose contribute to the formation of the ferment, d-mannose and rhamnose are inactive. In the active sugars the configuration

$$\begin{array}{ccccc} & \mathrm{OH} & & \mathrm{H} & \\ — & \mathrm{C} & — & \mathrm{C} & —\mathrm{CHO} \\ & \mathrm{H} & & \mathrm{OH} & \end{array}$$

or its optical antipode exists, whereas in the inactive sugars both hydroxyl groups are on the same side of the chain of carbon atoms.

II. *Specificity in the Use of Sugars*

By floating detached leaves, which have been deprived of their starch by keeping them in the dark, on nutrient solutions, it is possible to determine which substances can occasion the formation of starch. The application of this method to the carbohydrate alcohols affords an excellent illustration of the influence of configuration on the physiological properties. Plants which normally contain alcohols can utilize these and also glycerol to form starch; thus the OLEACEÆ utilize mannitol, *Lingustrum* and *Chieranthus* make use of dulcitol. The ROSACEÆ are able to produce starch from sorbitol, the production being more vigorous than from carbohydrates or from glycerol, but they are quite unable to utilize mannitol or dulcitol. The members of this group produce sorbitol, commonly in their metabolic processes. The leaves of *Adonis vernalis* are able to con-

vert adonitol into starch, but can make use of no other carbohydrate alcohols.

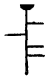
The three polysaccharides, sucrose, raffinose, and stachyose, may be regarded as fructose derivatives of increasing complexity. The invertase of beer yeast eliminates fructose from all of them.

In their action on optical isomers the enzymes have specific action. Some are able to use only aldohexoses of a certain configuration. Yet if the proper configuration is present, the action proceeds as if an inorganic catalyst were concerned. The same condition holds for the action of organisms on other optically active substances. In fact, the first case of this kind was discovered by Pasteur, who found that there were two forms of optically active tartaric acid, the dextrorotatory d- and the levorotatory l- forms, differing in their physiological properties as well as in their crystal form. *Penicillium glaucum* will not ferment the l- form of tartaric acid but does attack the d- form. From a mixture of the two, Pasteur was able to separate the l- form in pure condition by fermenting out the d- form with a culture of *Penicillium*. Also, with the microscope he picked out the corresponding d- and l- crystals from a mixture.

This same specificity for certain configurations of the molecule is shown for all of the sugars and for the alcohols or acids derived from them. For instance, there is a difference in the fermentation of mannitol and dulcitol by *Bacillus coli* which ferments mannitol and *Bacillus coli aërogenes* which ferments both. Many yeasts (*Saccharomyces sp.*) will ferment d-fructose but will not attack l-fructose. In general, d-glucose, d-mannose, and d-fructose are fermentable by most organisms, but l-glucose, l-mannose, and l-fructose are not attacked. Ordinary yeast will ferment the d- forms of these hexoses with about the same rate for all. But d-galactose, belonging to a different series from the d-glucose group, is not so easily fermentable by yeast. D-talose is related to d-galactose in the same way that d-mannose is related to d-glucose, but d-talose cannot be fermented by yeast. The difference in fermentability does not depend upon the position of the individual OH or H groups, but rather depends upon their relative position in the molecule.

Fischer explained the specificity of enzymes by the statement that the enzymes themselves show asymmetric molecular structure. The structure of the enzyme and its sugar substrate may be a complementary arrangement similar to that of lock and key. Of course this is merely an attempt to visualize the method of action and may not actually hold at all. We must know more about the enzymes before their specificity can be explained satisfactorily.

The action of yeast on sugar indicates that the yeast zymase has three active groups arranged for convenience in explanation in the follow-

ing order:  This arrangement enables it to attack the glucose molecule and disintegrate it. These active groups of the zymase would then correspond to the arrangement of the OH group in the sugar. With these groups it attacks the d-glucose molecule with the OH groups in a corresponding position. This enzyme can attack d-galactose with only two active groups. Hence d-galactose is less easily fermentable while d-talose is not fermentable at all.

zymase d-glucose d-galactose d-talose

II. *Configuration of Sugars in Relation to Their Use in Alcoholic Fermentation*

The mechanism of the formation of ethyl alcohol, C_2H_5OH, and carbon dioxide, CO_2, by the fermentation of d-glucose may be explained on the basis of labile hydrogen or hydroxyl groups. There is probably a series of reactions preceding the decomposition. It has been suggested that the first process in fermentation is the conversion of the sugar into the enolic form by means of an enzyme contained in the yeast. The three fermentable hexoses yield the same enolic form, but possibly it is formed at different rates according to the sugar; and whether one and the same agency is operative in each case it is impossible to say. The subsequent decomposition of the molecule is the same for each of the three hexoses, an hypothesis which is quite in agreement with experimental observations. This decomposition is also due to an enzyme or to several enzymes acting in turn. The breakdown of the molecule may commence at the double linkage beyond the carbon atoms in the 2–3 dienol.

This view is quite in harmony with the discovery by Harden and Young that the first stage in the fermentation of glucose by zymase is the formation of hexose phosphate $C_6H_{10}O_4(H_2PO_4)_2$. Glucose, mannose, and fructose give rise to the same hexose phosphate. When this hexose phosphate is hydrolyzed, fructose is obtained. In other words, the hexose phosphate may be regarded as a compound of the enolic form of the three hexoses.

Within the limits imposed by the conditions of the experiment, the addition of soluble phosphates to a fermenting mixture of a hexose with yeast juice or zymin causes the production of an equivalent amount of

CO_2 and alcohol. The phosphates we say act as coenzymes or are activators for zymin.

Hexose phosphoric acid, $C_6H_{10}O_4(H_2PO_4)_2$, is formed by d-glucose, d-mannose, or d-fructose, and it contains an active carbonyl group and two phosphoric acid groups.

Further support of this view of the fermentation process as afforded by the fact that substances so closely related to glucose as the methyl glucosides, glucosazone, gluconic acid, and ethyl gluconate are unfermentable without exception. In all these, only the groups attached to the terminal carbon atom differ from those of glucose. Enolization in them, however, is impossible, and no action takes place since the formation of hexose phosphate is prevented.

The behavior of galactose is altogether different. It is fermented with much greater difficulty than glucose. Very many yeasts are quite without action on galactose. The temperature coefficient of the fermentation of galactose is different from the value found in the case of glucose. These facts suggest that galactose is fermented by a different mechanism, that a different enzyme contained in galactozymase is concerned perhaps in causing enolization, which is less widely distributed in yeasts. None the less, the two phenomena must be very closely allied. No yeast is known capable of fermenting galactose but not fermenting glucose.

The change in configuration in passing from glucose to galactose, though not sufficient to prevent fermentation altogether, causes the compound to be far more resistant to attack. It is not surprising, therefore, that any further change in configuration is sufficient to make the new hexose no longer fermentable. This is illustrated by the behavior of galactose and its isomers, talose and tagatose, which have an enolic form common to all three hexoses.

CHO	CHO	CH_2OH	CHOH
|	|	|	‖
HCOH	HOCH	C=O	COH
|	|	|	|
HOCH	HOCH	HOCH	HOCH
|	|	|	|
HOCH	HOCH	HOCH	HOCH
|	|	|	|
HCOH	HCOH	HCOH	HCOH
|	|	|	|
CH_2OH	CH_2OH	CH_2OH	CH_2OH
d-galactose	d-talose	d-tagatose	enolic form

Neither d-talose nor d-tagatose is fermented by any yeast whose action toward them has at present been investigated. Yet in d-talose the position of the two upper hydroxyl groups is the same as that in d-mannose, and the lower three hydroxyls occupy the same positions as they do in d-galactose. Obviously, for it to be fermentable the configuration of the hexose has to be correct as a whole, the fact that single hydroxyl groups occupy the same positions as they do in fermentable hexoses being of no moment. Presumably yeasts contain no enzymes compatible with talose or tagatose and which are able to convert them into the enolic form.

The facts described can only be explained on the assumption that there is the very closest relationship between the configuration of a fermentable hexose and the enzymes which cause fermentation. This hypothesis receives confirmation which is little short of absolute when the behavior of the sugars other than the hexoses is considered. No pentose, either natural or synthetic, is fermentable by yeast. None of the synthetic tetrose, heptose, or octose carbohydrates are fermentable.

The only fermentable sugars, other than the four hexoses, are a nonose prepared by the cyanohydrin method from mannose, and a ketotriose, dihydroxyacetone. Pure dihydroxyacetone is fermented by very active yeasts.

A further illustration of the relation of configuration to fermentability is afforded by the behavior of that monomethylglucose in which the methoxyl group is attached to the carbon at the extreme end of the chain and therefore most remote from the part of the sugar molecule which is generally believed to have the most effect in controlling enzyme action.

$$\mathrm{MeO{-}CH_2{-}CHOH{-}\overbrace{CH{-}(CHOH)_2{-}CHOH}^{O}}$$

Living top yeast and a maceration extract of dried bottom yeast were quite without action on this monomethylglucose. The compound also resisted seven species of bacteria, all of which acted on glucose.

The identification of intermediate products in the fermentation of glucose has long been a matter of controversy. Buchner and his coworkers have suggested in turn lactic acid ($CH_3{-}CHOH{-}COOH$) and dihydroxyacetone ($CH_2OH{-}CO{-}CH_2OH$) as intermediates, but in both cases Slator has shown that these fermented very much more slowly than glucose, an observation which renders Buchner's hypothesis doubtful. The same will probably apply to the suggestion that formic acid is the intermediate product. Bearing in mind Fischer's synthesis of acrose

from dihydroxyacetone, it appears probable that dihydroxyacetone is fermented by yeast only after it has been converted into hexose, and the same applies to glyceric aldehyde. This hypothesis is greatly strengthened by Lebedeff's proof that the organic phosphate produced during the fermentation of dihydroxyacetone is identical with the hexose phosphate obtained by Harden and Young from the fermentable hexoses. It is probable, therefore, that dihydroxyacetone is fermented only after conversion into hexose.

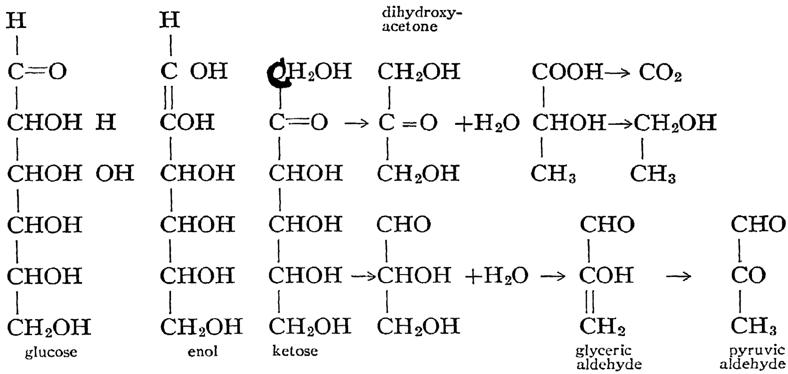
Evidence is accumulating, however, that pyruvic acid, $CH_3—CO—CO_2H$, is a normal intermediary in fermentation. When yeast is grown in sugar solutions in presence of sodium sulphite, considerable quantities of acetaldehyde are formed. This fact is the basis of the suggestion by Neuberg that the sugar breaks down into two molecules of pyruvic acid which are rapidly converted into aldehyde by the yeast carboxylase. The aldehyde in turn acts as an acceptor of hydrogen and promotes the formation of pyruvic acid from sugar under the influence of the yeast reductase, half the aldehyde being at once converted into alcohol.

It is obvious how intimately the property of undergoing fermentation is connected with the configuration of the sugar molecule. Lengthening or shortening the chain of carbons is sufficient to place the sugar molecule out of harmony with the yeast enzymes, and thus prevent its destruction by fermentation. The fact that triose, hexose, and nonose sugars are fermentable has led to the suggestion that the fermentable carbohydrates must contain a multiple of three carbon atoms; possibly three carbon chains are intermediate in the fermentation, but the fermentability of the nonose requires confirmation.

Although hexose phosphate is formed under the influence of yeast juice, living yeast cells do not ferment it, even when added coferment and artificial activators are supplied. Dried yeast or yeast juice esterifies phosphate almost quantitatively in the presence of glucose, whereas living yeast, even when toluene has been added, may esterify only some 8%; the difference is probably a question of cell permeability. It is further of interest that some yeasts, when weakened by nitrogen starvation, are able to esterify phosphates in presence of fructose but not with glucose. This is an indication that the protoplasm can grip the ketose structure more readily than the aldose structure and that the preparatory process in fermentation may be concerned in the conversion of aldose into ketose, or far more probably into a common enolic or oxide form, which is more easily formed from fructose than from glucose.

In this connection it is common knowledge that fructose is usually more easily or better utilized in the animal body than glucose, as, for example, under diabetic conditions.

Lactic acid may be an intermediate in the formation of ethyl alcohol and CO_2 from glucose. Yeast juice incubated with glucose contains small quantities (.2%) of lactic acid. Increasing the rate of fermentation by a strong zymase preparation increases the amount of lactic acid. The addition of glucose or lactic acid favors the decomposition of the lactic acid. Two enzymes play a part in the reaction, one producing the interchange of position which leads to the formation of lactic acid, the other effecting the decomposition of lactic acid into ethyl alcohol and CO_2.



Glucose and fructose probably play different parts in metabolism. Glucose is mainly concerned in respiration; fructose appears to take part in the elaboration of tissue compounds since it is far less stable than glucose. Yeast and molds for equal weights of sugar consumed show greater growth on fructose. They respire glucose preferentially from invert sugar. Fructose may yield residues of varying chain length for the synthesis of the great variety of compounds found in protoplasm. The structural parts of plants also contain galactose anhydrides, and glucose anhydrides are the principal energy-storage forms.

CHAPTER VII

CERTAIN SUBSTANCES DERIVED FROM SUGARS

I. *Synthesis of Higher Sugars and Related Alcohols*

The cyanohydrin synthesis of sugars is of interest to the physiologist as well as to the organic chemist, on account of its possible biological connections. This method is used for the synthesis of sugars of a greater number of carbon atoms from sugars already prepared. There is a condensation of the hydrocyanic acid with the aldehyde group of the sugar to form the cyanohydrin. On hydrolysis the cyanohydrin then yields an hydroxy acid and ammonia as follows:

$$CH_2OH(CHOH)_4CHO + HCN \rightarrow CH_2OH(CHOH)_4\begin{array}{l} \;/H \\ C\text{——}OH \rightarrow \\ \;\backslash C{\equiv}N \end{array}$$

$$CH_2OH(CHOH)_4\begin{array}{l} \;/H \\ C\text{——}OH + NH_3 \\ \;\backslash C{=}O \\ \;\;\;| \\ \;\;\;OH \end{array}$$

There has been formed by this process an organic acid containing one more carbon atom than the sugar from which it was made. On reduction to the aldehyde, this acid will yield a sugar of a correspondingly increased number of carbon atoms.

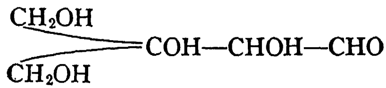
Hydrocyanic acid occurs in plants and it is frequently found in glucosides. Possibly reactions of this nature are concerned in the synthesis of heptoses or even more commonly occurring sugars. From these higher sugars there may be derived various higher alcohols occurring in plants.

Several carbohydrate alcohols occur widely distributed in plants. Erythritol is found in many mosses and algæ. Adonitol is found in *Adonis vernalis*. D-mannitol is found in manna, the sap of larch, and in many fungi. It is probably derived from trehalose by bacterial action. It forms mannitol, an incrustation over the thallus of *Laminaria*. D-sorbitol is

present in the fruits of most of the ROSACEÆ and also in the leaves. It forms a crystalline inflorescence on the stipes of *Boletus bovinus*. L-iditol is present in mountain-ash berries. L-dulcitol occurs particularly among the SCROPHULARIACEÆ.

The seven-carbon alcohol perseitol occurs in Persea gratissima, the avocado; and volemitol is found in *Lactarius volemus* and in the rhizomes of numerous species of *Primula*. An octitol is found in some ROSACEÆ. All these alcohols may be produced by reduction of the corresponding aldose sugars, yet in plants the corresponding sugars are not very abundant. A heptose, mannoketoheptose, occurs in the avocado-pear (*Persea gratissima*). Sedoheptose occurs in *Sedum spectabile*. It is not fermentable.

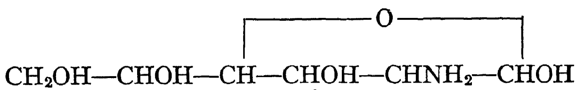
The sugar apiose has the unusual formula:



It occurs in apiin, a glucoside found in a number of plants of the family UMBELLIFERÆ. Isovaleric acid may be produced from this sugar, and there is probably a relation between the presence of this sugar and the isovaleric acid found in some of the UMBELLIFERÆ.

II. *Aminohexoses*

D-glucosamine is found in chitin in the walls of fungi. It is present in *Boletus edulis* and *Lycoperdum gematum*, as well as in the shells of crustaceans and insects.

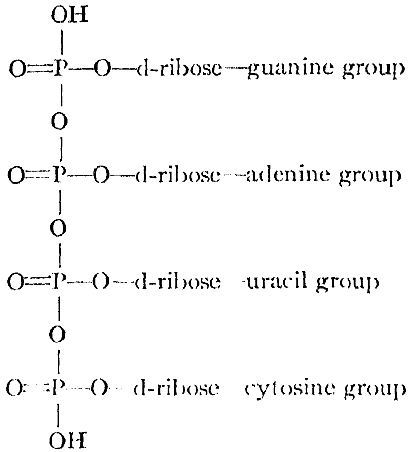


glucosamine

This substance is intermediate in composition between the amino acids and the carbohydrates. It may be of some importance in metabolic reactions but usually is found only as its anhydride, chitin, an unreactive substance of the fungus wall.

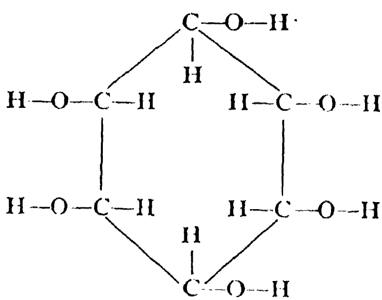
Phosphoric acid esters of carbohydrates are important in the structure of the nucleic acids. Inosinic acid on hydrolysis yields a purine base and d-ribose phosphoric acid. Yeast nucleic acid contains d-ribose phosphoric acid.

Plant nucleic acids are built up on the following scheme:



The phosphoric esters are important constituents of the nucleus. All the plant nucleic acids are identical as far as known. The uracil group is found only in plants, being replaced in animals by thymine. The nucleic acids undoubtedly play an important part in the assimilation of nitrogen, in the synthesis of protein, in cell growth, and in respiration.

Inositol ($C_6H_{12}O_6$, a cyclic compound) occurs in the LEGUMINOSÆ, in leaves of asparagus, oak, ash, and cereus, and in oily seeds such as walnuts. It is found also in mistletoe, in all parts of the grapevine, and in many fungi. Phytin, the ester with phosphoric acid, is found in many seeds. These ring compounds may be produced by the union of the end carbons of the carbohydrate chain.



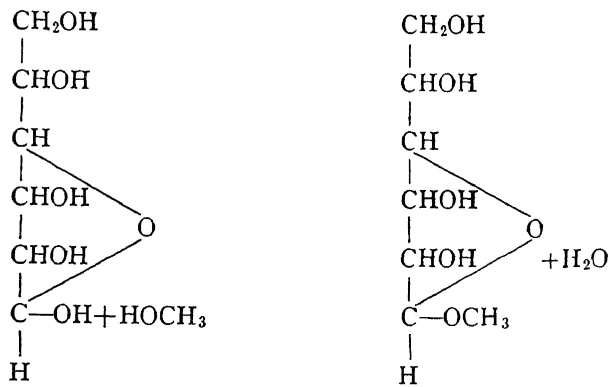
Hexahydroxybenzene, or inositol

CHAPTER VIII

GLUCOSIDES

Both animals and plants have the property of removing injurious substances by combining them with glucose to form glucosides. Thus, when camphor is taken into the animal circulation, it is combined with glucose to form a glucoside. The glucose molecule in such combinations can still be oxidized at the alcohol end to form glucuronic acid, in which form the combination is removed by excretion from the kidney in animals. A great many substances, including phenols and constituents of volatile oils, are thus paired with glucuronic acid and excreted by animals. Possibly the glucosides of plants are formed in a similar manner, and their formation may be for a similar purpose.

The glucosides are ether-like compounds of carbohydrates with other bodies or with other sugars. More strictly speaking, the term *glucoside* may be applied to such compounds formed by glucose alone, but the term is generally not so restricted. Upon hydrolysis, glucosides give glucose as one product. Disaccharides, containing glucose, in general may be considered glucosides, but the name is usually restricted to combinations of glucose and compounds other than sugars. The glucosides exist quite commonly in plants. As an example of the formation of a glucoside the following reaction may be given:



β-d-glucose β-methyl glucoside

The following substances may enter into combination with glucose to form glucosides:

Other sugars, example sucrose	Oxanthraquinone
Phenols	Oxyflavone derivatives
Alcohols	Indoxyl
Aldehydes	Mustard oils
Acids	Various other compounds
Oxycumarin derivatives	Saponins, digitonins, etc.

The amount of glucoside present varies considerably in different species of the same genus and varies at different seasons of the year. The glucosidal content differs in the male and female plants of the same species. Jowett and Potter in thirty-three samples of bark of willow and poplar found a considerable variation in the amount of salicin. In April the bark of the female tree contained three times as much as the male. Three months later the condition was reversed. It has been suggested that salicin acts as a reserve, being stored away in the winter and used up in the spring. Taxicatin, found in *Taxus baccata*, is most abundant in fall and winter, but this condition does not hold for sambunigrin, an isomer of mandelonitrile glucoside found in *Sambucus niger*. Sambunigrin remains in the leaves of the elder when they fall and does not migrate to the stem as many glucosides do. Hydrocyanic acid of glucosidal origin is abundant in the seedling stages in many plants. As the plants become older the cyanogenetic glucosides may in some cases increase or in others disappear. The amount of cyanogenetic glucoside in plants sometimes can be controlled by the cultural methods for the crop; high nitrates generally favor the production of cyanogenetic glucosides.

The cyanogenetic glucosides are common in young grass plants of certain species. They are frequent causes of poisoning in live stock. They are especially abundant in dry weather or if the nitrate supply of the seedlings is great. Gautier believed HCN arose from the action of formaldehyde on nitrates. Treub and others suggested that HCN is a step in the reduction of nitrate to (NH_2) the amino group, a step in protein synthesis; but this is not fully established. The hemolytic action of glucosides depends upon their ability to unite with cholesterol. They have the same power to unite with the phytosterol of plants.

A very common glucoside is amygdalin. It is found in bitter almonds, in peach seeds, raisin seeds, lemon peel, etc.

$$_{1}N + 2H_2O \xrightarrow{\text{maltase}} C_{14}H_{17}NO_6 + C_6H_{12}O_6$$

in

mandelo-
nitrile
glucoside

$$C_{14}H_{17}NO_6 \xrightarrow{\text{emulsin}} C_7H_{16}O + HCN + C_6H_{12}O_6$$

mandelo-nitrile glucoside — benzal-dehyde — glucose

The hydrolysis of amygdalin is carried on by emulsin. The glucosides and their hydrolytic enzymes usually are found in separate cells in the

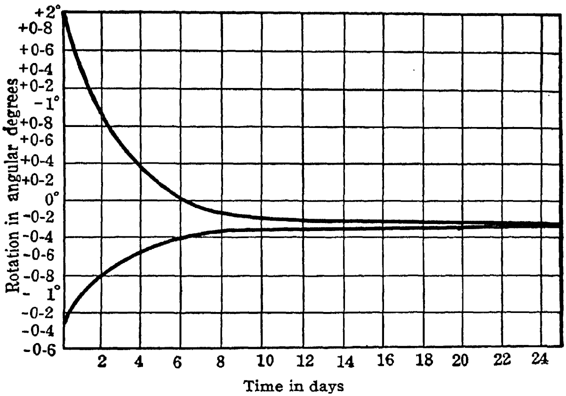


FIG. 29.—Graph showing the relation between the time in days and the angle of rotation in degrees as a measure of the hydrolysis or synthesis of β-glycerin glucoside by emulsin. Both mixtures contained equivalent quantities of glycerin, glucose, and water in the lower curve, and of glucoside in the upper curve, taken with the same quantity of emulsin. (After Bayliss.)

living plant and mix only on crushing the cells. Emulsin has both synthetic and analytic action, as shown in Fig. 29.

Another type of glucosides, the mustard oil glucosides, contain sulphur. The sinigrin of mustard seeds is an example of this type. On hydrolysis, sinigrin decomposes as follows:

$$\underset{\text{sinigrin}}{C_{10}H_{18}O_{10}NKS_2} + H_2O \rightarrow KHSO_4 + \underset{\text{mustard oil}}{C_3H_5CNS} + C_6H_{12}O_6$$

$$\underset{\text{allyl isothiocyanate}}{CH_2{:}CHCH_2NCS}$$

The hydrolytic enzyme acting upon sinigrin is not emulsin but myrosin. The enzymes and glucosides are brought together upon injury of the cells, as by crushing. It may be noticed that the strong flavor of the onion may be accentuated after crushing. But on long standing the crushed tissue loses its flavor.

There is another class of glucosides of importance for plants, the chromogen-producing glucosides of which indican is an example. A dye, indigo, is formed from indican by hydrolysis and oxidation.

$$\underset{\text{indican}}{C_{14}H_{17}O_6N} + H_2O \text{—} C_6H_{12}O_6 + \underset{\text{indoxyl a chromogen}}{O_8H_7OH}$$

$$2\underset{\text{indoxyl}}{C_8H_7OH} + O_2 \text{—} 2H_2O + \underset{\text{indigotin, or indigo blue}}{C_{16}H_{10}O_2N_2}$$

Dipsacan, contained in all plants of the family DIPSACACEÆ, is another example of the glucosides which produce chromogens on hydrolysis. Dipsacan occurs abundantly in *Baptisia tinctoria*, commonly called wild indigo.

According to Palladin the chromogen-producing glucosides are almost universally distributed. He considered them to be of great biological importance because they are connected with the activity of the plant in respiration. They probably function as oxygen carriers in oxidative processes. The anthocyanins are related to the glucosides and may have similar functions. Anthocyanin formation depends upon the presence of glucose. Generally they are found more abundantly under conditions which produce high concentrations of glucose in the cells, such as low temperature exposure, etc.

There are many other glucosides in plants of which a few may be mentioned. Arbutin, found in *Arbutus sp.*, is hydrolyzed by emulsin or mineral acids, giving glucose and hydroquinone.

$$C_{12}H_{16}O_7 + H_2O \rightarrow C_6H_{12} + \underset{\text{hydroquinone}}{C_6H_6O_2}$$

Salicin is found in the bark of willow (*Salix sp.*). Emulsin or mineral acids cause its hydrolysis to saligenol and glucose.

$$\underset{\text{salicin}}{C_{13}H_{18}O_7} + H_2O \text{—} \underset{\text{saligenin or -ol}}{C_7H_8O_2} + C_6H_{12}O_6$$

Saligenol upon oxidation gives salicylic acid $C_6H_4\langle{}^{OH}_{COOH}$ All of these substances may be demonstrated in the willow.

The function of glucosides in plants is an open question. However, they are to be considered as reserve foods since they contain glucose. They may be harmless compounds formed as in the animal body from by-products of reactions which might be injurious otherwise. They may also serve as a protection to the organisms. They are hydrolyzed upon the death of the cells or upon treating the tissue with ethylene and similar compounds. The products of the hydrolysis of glucosides may serve as antiseptics upon the injury of an organ. In the bark of trees these anti-

septics may tend to prevent the growth of parasites. Amygdalin yields benzaldehyde, arbutin yields hydroquinone, and these substances inhibit the growth of many fungi.

The saponins are a class of substances related to the glucosides. They are rather widespread in plants. In general they are quite toxic to animals, and they are found in great quantity in some plants. Yucca rhizomes contain as much as 20% saponin. Such poisonous substances protect the plants from being eaten by animals. Solanin, a glucoside found in the family SOLANACEÆ, has been reported to be of importance in the immunity of potatoes to dry-rot. Onion smut resistance has been correlated with the presence of glucosides in certain varieties.

The glucosides may have a regulatory function in metabolism. Possibly the formation or decomposition of glucosides may regulate the rate of respiration by removing oxygen carriers from activity or by increasing the quantity of oxygen carriers. Several glucosides are definitely known to be of importance in respiration since they yield oxygen acceptors such as hydroquinone.

The fructosides are very easily hydrolyzed in comparison with glucosides; this may account for the fructosides not being of as common occurrence in plants as the glucosides.

CHAPTER IX

DISACCHARIDES, TRISACCHARIDES, AND TETRASACCHARIDES

I. *Disaccharides*

The disaccharides are of two types, reducing and non-reducing. The reducing disaccharides contain free carbonyl groups. The carbonyl groups of the non-reducing disaccharides are bound.

The common disaccharides and their composition are as follows:

		Reducing			
	Maltose	→	d-glucose		d-glucose
	Lactose	→	"		d-galactose
	Melibiose				d-galactose
	Turanose			+	d-fructose
Formed by hydrolysis of tri-saccharides	Gentiobiose				d-glucose
	Cellobiose				d-glucose
		Non-reducing			
	Sucrose		d-fructose		d-glucose
	Trehalose		d-glucose		d-glucose

There is some interest in knowing that pentose and hexose sugars exist together as disaccharides, since this gives evidence for the origin of pentose sugars from dihexoses with subsequent hydrolysis or from polyhexoses. Pentoses and hexoses exist in combination also in polysaccharides and in some glucosides.

We know of no really characteristic derivatives of the non-reducing disaccharides by which we may isolate and identify them. Therefore the study of the synthesis of disaccharides in plants is rather difficult.

I. MALTOSE

Maltose is found in plants in very small amounts. It yields only d-glucose on hydrolysis by maltase or by mineral acids. Although it was at one time generally stated that maltase did not usually occur in plants, W. A. Davis gives strong reasons for supposing that it is always present at least where starch degradation occurs Maltase is endocellular in origin, and it is readily destroyed by temperatures above 50°. It has low solubility in water and low powers of diffusion. Daish has identified maltase in the crushed pulp of a number of leaves, all of which convert gelatinized starch into glucose.

Maltose can be oxidized to a monobasic acid, maltobionic acid, $C_{12}H_{22}O_{12}$, which on hydrolysis splits into d-glucose and d-gluconic acid. This indicates that there is one free aldehyde group in the maltose molecule. Maltose then must not yield pentoses on oxidation as the other disaccharide sugars may do, because this oxidation would yield a configuration of the pentose not found in plants. Maltose forms an osazone with two, not four, molecules of phenylhydrazine; hence it has only one carbonyl group of the two glucose molecules uncombined. The union of glucose in maltose involves only one carbonyl oxygen. Such a linkage is called a *monocarbonyl bond.* Maltose is not a storage form because it does contain a reactive carbonyl group.

2. SUCROSE

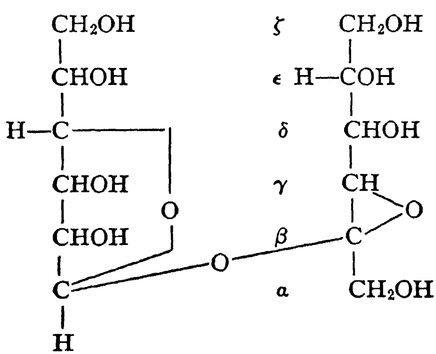
Sucrose, or cane-sugar, is commercially the most important of the sugars. It is widely distributed in the plant kingdom. It functions chiefly as a reserve food, since it has no reactive carbonyl group. It is very soluble in water. Sucrose crystallizes out from solution with ease in comparison with the monosaccharides. Doubtless this is due to the fact that there is no mixture of isomerides present when the disaccharide is in solution in water.

Sucrose does not reduce Fehling's solution; hence it has no free carbonyl group. Such a junction is called a *dicarbonyl bond.*

$C_6H_{11}O_5$

d-glucose d-fructose

Sucrose does not exhibit multirotation. The union of the glucose and fructose in sucrose may be represented by the following formulæ:



d-glucose d-fructose

Sucrose

Sucrose lacks both aldehyde and ketone characteristics. It forms no compounds with phenylhydrazine, and it is stable toward alkalies. It contains eight hydroxyl groups, which is proven by the formation of an octa-acetate and octa-methyl derivative.

3. HYDROLYSIS OF SUCROSE

Sucrose can be hydrolyzed by mineral acids to glucose and fructose. It can be split by invertase or sucrase, an enzyme of wide occurrence. Fig. 30 shows the relation of the acidity of the medium to the activity of invertase.

In the cell the inversion of sucrose is accomplished not so much by H^+ as by the action of sucrase. Nevertheless, the cell sap acidity is important because the activity of sucrase itself is quite markedly affected by the H^+ concentration of the medium within the range of acidities found in plant tissues. In the plant, then, where sucrase is present, the rate of sucrose cleavage or inversion is not the direct proportionality shown in solutions of H^+ and sucrose only. In the region from pH 3 to pH 4 the rate of hydrolysis will be very remarkably affected by the acidity, while in the range from pH 4 to pH 6 the rate will not be so greatly affected by change in acidity because the curve (Fig. 30) in this range is rather flat, that is, the effect of H^+ on the activity of the sucrase (invertase) is not so remarkable as in the range pH 3-4. On the alkaline side of neutrality there is a remarkable effect of the H^+ concentration on the activity of sucrase, yet the change in H^+ concentration itself is not such as to be of much importance in affecting the inversion.

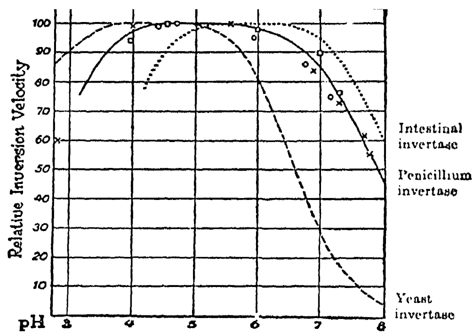


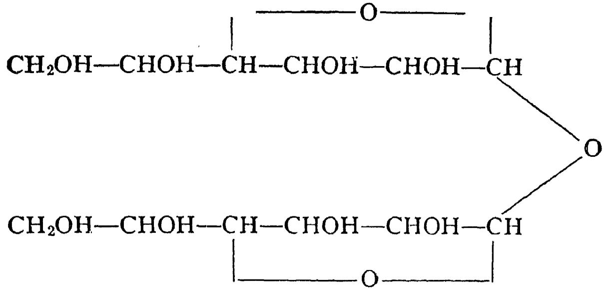
FIG. 30. Influence of reaction upon the activities of three typical invertases. (After Euler et al.)

Sucrose is not directly fermentable; it is first hydrolyzed and then the glucose and fructose can be fermented. Yeasts that do not contain invertase cannot ferment sucrose, for example, *Saccharomycse octosporus* does not attack it.

4. TREHALOSE

Trehalose occurs widely distributed in fungi. It is composed of two glucose molecules fused in such a way that both reactive alde-

hyde groups have disappeared. The linkage may be represented as follows:



This structure of trehalose is indicated by the fact that it does not reduce Fehling's solution or form osazones, or show mutarotation. Trehalose is not affected by enzymes such as maltase, invertase, emulsin, or diastase, but has its specific hydrolytic enzyme, trehalase. Trehalase is found in certain fungi and many species of yeasts. It may be conveniently obtained from *Aspergillus niger*.

Trehalose is hydrolyzed with difficulty by acids, differing from sucrose markedly in this regard. Apparently trehalose replaces sucrose in those plants, the fungi, which contain no chlorophyl and which do not manufacture starch. The storage of trehalose in fungi is at a maximum just before spore formation. When the fungi are picked, trehalose is first hydolyzed and then the glucose is rapidly changed to mannitol.

5. LACTOSE

Lactose is of interest mainly as a substrate for the growth of fungi and bacteria. Lactose shows mutarotation. It reduces Fehling's solution and forms an osazone. It exists in isomeric forms in solution in water. The experimental evidence shows the potential aldehyde group to be in the glucose part of the molecule. Lactose is hydrolyzed by its specific enzyme, lactase. Lactase is found in a few yeasts and in the crude emulsin extracted from almonds. Lactose is particularly likely to undergo hydrolysis with subsequent formation of lactic acid by fermentation, as by *Bacillus lactis*.

II. *Trisaccharides*

Raffinose is the best known trisaccharide. It is often found in considerable abundance in the sugar-beet, and also is present in other plants. By strong mineral acids it is hydrolyzed completely to glucose, fructose, and galactose. By dilute acids melibiose and fructose are formed. By

invertase it is hydrolyzed to melibiose and fructose. Emulsin hydrolyzes it to sucrose and galactose. This series of actions gives an excellent insight into the points of action of those substances and the type of linkage upon which they act. Bottom yeasts are able to hydrolyze raffinose completely, for they contain invertase and melibiase, the enzyme which hydrolyzes melibiose.

Raffinose has no direct reducing power. It behaves chemically much like cane-sugar. It may be represented by the following formula:

$$\underset{\text{fructose}}{C_6H_{11}O_5}\underset{\text{sucrose}}{—O—}\underset{\text{glucose}}{C_6H_{10}O_4}\underset{\text{melibiose}}{—O—}\underset{\text{galactose}}{C_6H_{11}O_5}$$

All of the linkages in raffinose are dicarbonyl linkages leaving no free carbonyl groups.

Melezitose is made up of d-glucose and d-fructose. It is not a reducing sugar, hence has no free carbonyl groups. It is found in the western larch (*Larix*) and in manna (*Fraxinus sp.*). On hydrolysis melezitose yields glucose and turanose. Turanose yields glucose and fructose on hydrolysis.

Gentianose yields fructose and two molecules of d-glucose. It is a non-reducing sugar. Gentianose yields fructose and gentiobiose by dilute acid hydrolysis. *Aspergillus* hydrolyzes it into glucose and sucrose.

III. *Tetrasaccarides* $C_{24}H_{42}O_{21}$

Stachyose is a tetrasaccharide. It may be obtained from tubers of *Stachys tuberifera*. When boiled with dilute mineral acids it gives d-glucose, d-fructose, and two molecules of d-galactose.

Lupeose, a tetrasaccaride commonly prepared from *Lupinus luteus*, is quite widely distributed in legume seeds.

CHAPTER X

POLYSACCHARIDES

The polysaccharides are characterized as carbohydrates of high molecular weight. They are mostly amorphous substances insoluble or slightly soluble in water. Like the disaccharides and trisaccharides they break up on hydrolysis into sugars of 5 and 6 carbon atoms; therefore, they may be considered anhydrides of these sugars. In the absence of exact knowledge as to the molecular weights, their formulæ are written $(C_6H_{10}O_5)_n$ or $(C_5H_8O_4)_n$, depending upon the constituent hexose or pentose sugars which they yield. They owe their importance to the fact that they include both storage material and structural materials of the plant. They make up the principal plant framework. They are present usually in admixtures, and their separation and purification in the natural condition is difficult.

I. *Classification of Polysaccharides*

1. Hexosans.
    - a. Dextrosans.
        - Dextrins.
        - Starches.
        - Normal celluloses.
        - Lichenin.
        - Dextran.
        - Glycogen.
    - b. Levulosans—inulin.
    - c. Mannosans—mannan.
    - d. Galactosans—galactan.
2. Pentosans.
    - a. Xylan.
    - b. Araban.
3. Gums, mucilages, and pectic substances.

II. *Starch*

Starch is one of the most widely distributed substances in the plant kingdom. It is the principal storage form in seeds, fruits, tubers, roots, and stems. It forms 50–70% of the dry weight of grains and 15–30% of the dry weight of potatoes. The starch content of leaves fluctuates with day and night. Starch is stored temporarily in leaves during the day,

then reconverted to glucose at night and translocated to other parts of the plant. Translocation during the day occurs normally, but the rate of synthesis in sunlight is more rapid than translocation. When continually illuminated by artificial light, starch comes to represent $^1/_3$ or more of the dry weight of some leaves, and finally a concentration is reached at which transformation and removal equals the rate of synthesis. There is then no periodic daily fluctuation of starch content, but each form of carbohydrate appears at an equilibrium concentration.

Starch grains show specific variation in shape and size. They are used as a means of detecting the adulteration of foods and drugs. Reichert determined the specific differences of starches in form and in physical and chemical properties.

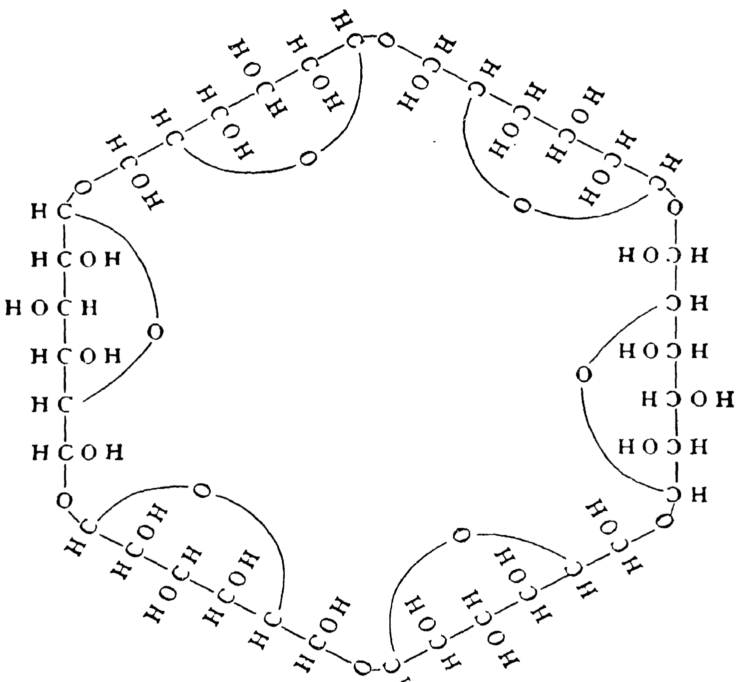
III. *Deposit of Starch*

Starch grains are deposited within the stroma of leucoplasts. Leucoplasts are specialized parts of the cell rich in protein and similar to chloroplasts. Leucoplasts may turn into chloroplasts on exposure to light as in the skin of the potato tuber. On photosynthesis starch deposits in or around the chloroplasts in many cases. Leucoplasts then may be regarded as changed chloroplasts lacking chlorophyl. The factors which determine the deposit of starch in leucoplasts are known incompletely, but deposit is usually explained as resulting after some accumulation of soluble carbohydrates. Certain leucoplasts function to produce starch deposition before others, causing starch accumulation in certain parts of the plant. We may explain this on the basis of a lower threshold value for starch deposit in these leucoplasts, but the reason for this is unexplained. Storage may occur almost anywhere in the plant. Quite frequently storage is limited to a small part of the plant, as in the remote end of a rhizome. Sometimes starch deposition seems to be started by injury, as in rhizomes of *Apocynum*. Similar cause has been stated to account for the formation of sweet potatoes.

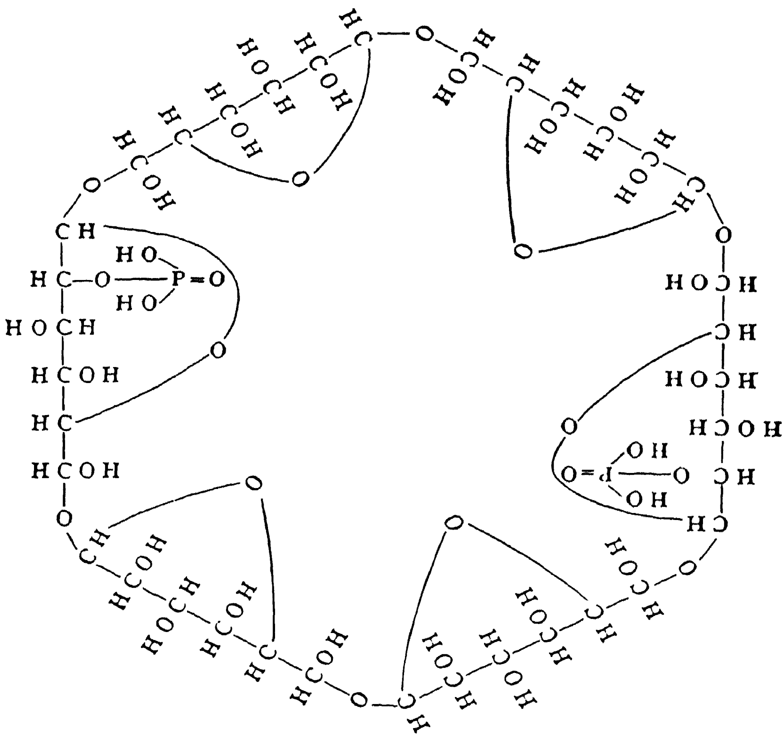
IV. *Starch Deposition in the Leucoplast*

The translocation of glucose and fructose from photosynthesizing regions causes accumulation in the storage organ until a threshold concentration is overstepped. Then a considerable quantity of starch is rapidly formed in the leucoplast. This forms a mass at the center of the starch grain different in water content from the rest of the grain. On drying of the grain this mass breaks, producing characteristic cracks referred to as the *hilum*. In daylight starch deposition goes on as long as synthesis and translocation occur. Under conditions of little synthesis or translocation the deposit has a different water content and probably also the molecules have time to arrange themselves in crystal forms.

During rapid deposition the starch formed within the leucoplast seems mostly to be amorphous and somewhat different in water content from the deposit at night. This causes differences in refractive index so that the starch grains appear banded. Each band then represents the amount of starch deposited during the day and night. In latitudes which differ in the length of day and night, one would expect to find differences in the starch deposits. If plants are continually illuminated the deposit is uniform and the bands disappear wholly or decrease in brilliancy. Monocotyledonous plants like wheat, which operate at high soluble carbohydrate concentrations, seem less sensitive to fluctuations in the rate of photosynthesis than those dicotyledonous plants such as potato which operate at low soluble carbohydrate content. The high soluble carbohydrate content has the effect of stabilizing the rate of starch deposition so that the bands become indistinct. A good example of this difference can be seen in comparison of wheat starch with potato starch grains.



Structure of starch molecule (amylose). (After Irvine.)



Structure of starch (amylopectin). (After Irvine.)

If deposition within the leucoplast starts simultaneously at two or more points, a compound starch grain is formed. The two points of deposition may fuse on stretching of the leucoplast as is the case of potato starch grains with a double hilum. In some cases the deposits never fuse and the grain is then made up of a number of polyhedral small grains, as in oat starch or rice starch.

In many monocotyledonous plants starch is mostly absent; for example, starch is not the storage form in *Scilla nutans*, *Pfleum pratense*, or *Allium*. In these species starch may occur in stomatal guard cells and a few other tissues in a small amount. In some other monocotyledonous plants such as *Musa*, starch deposition begins only when much sugar

has accumulated. In other monocotyledonous plants such as *Lilium tigrinum* and *Tradescantia virginica* starch is regularly present. In *Scilla nutans* starch is absent, while in the closely related *Scilla sibirica* starch is present.

V. *Composition of Starch Grains*

When air dry, starch contains as much as 10–20% of water, depending upon the relative humidity. The greater part of this water can be removed by careful heating to 100° C. When starch is heated to about 200° C. chemical changes occur forming a mixture of substances of the empirical formula $C_6H_{10}O_5$. British gum or dextrin is formed in this manner. Starch grains are insoluble in cold water. When heated with water the starch grains swell and burst, to form an opalescent mass, starch paste. The consistency of the paste varies with the concentration, and also the kind of starch. When a heated solution of starch is filtered, a colloidal solution of starch is obtained, known as soluble starch. Soluble starch exists in some plant saps, and part of the starch grain in some plants may be dissolved in water.

Starch grains contain approximately 80–85% amylose and 15–20% amylopectins. Soluble starch (starch granulose) produces a blue color with iodine. It is easily digested by malt diastase. Insoluble starch gives no blue color with the iodine test and is more resistant to maltase digestion. There are contained in the starch grain also paste-forming substances which are very resistant to maltase action.

VI. *Composition of Various Starches*

All starches contain amylopectin and various amyloses in different proportions. The different starches may also be distinguished from one another by the effect of water on the amylopectin and by the solubility of the amyloses in hot water. The differences in these physical and chemical properties of starches is given in great detail by Reichert and by Tanret.

Starch	*Amylose*	*Amylopectin*
Oats	71.5	28.5
Banana	79.5	20.5
Wheat	67.5	32.5
Maize	70	30
Barley	73	27
Pea	78.5	21.5
Rye	78.5	21.5
Potato	73	27
Rice	68.5	31.5
Apple	76	24

VII. *Ideas of the Structure of the Starch Grain*

Nageli thought that starch grains were made up of prismatic micellæ in a matrix of non-crystalline material, and that the lamellæ or rings were due to differences in water content. Schimper and Meyer considered the grain to be a sphærocrystal of α- and β-amylose. Kraemer demonstrated crystals and colloids both at the hilum and in alternate lamellæ. Many others hold this same view of the structure of starch grains.

VIII. *Chemical Tests for Starch*

The principal microchemical reagent for starch is iodine. In the presence of hydriodic acid, or alkaline iodides, a blue adsorption compound is produced by starch. The blue color disappears on heating but returns on cooling. In alcohol or in the presence of formaldehyde the blue color is not produced. The blue color is intensified owing to swelling of the grains when they are treated with chloral hydrate, alkalies, or boiling water. A glucoside, saponarine, found in the epidermis of leaves of 24 species in 8 families of flowering plants and 1 species of liverwort gives the same color reaction with IKI that starch gives. Some starch grains turn red with iodine. Examples of this reaction are found in the root cap of *Allium sepa*, in the seed coats of *Oryza sativa*, or in *Chelidonium sp.* In these tissues the color difference is due to dextrin and amylodextrin.

There has been a long controversy as to whether the starch iodine color reaction was due to chemical union or to physical adsorption. According to Harrison's explanation, the color is due to a colloidal solution of iodine in starch.

IX. *Soluble Starch*

On heating starch with water alone, or heating with glycerin to 190° C., or on treating with 6 parts of HCl (sp. gr. 1.06) at ordinary temperature for 6 to 8 weeks, it is converted into soluble starch or amylodextrin. Soluble starch is also formed as an intermediate step in the conversion of starch into sugar by dilute acid or diastase.

Soluble starch may be obtained by pouring a 1–2% aqueous solution, prepared by heating purified starch, into a large excess of pure acetone and shaking it vigorously. A flocculent precipitate is thus formed. This is filtered and ground in a mortar with more acetone. When dried *in vacuo*, a light white powder completely soluble in cold water is produced which is known as *soluble starch*.

X. *Action of Acids on Starch*

The digestion of starch with 12% HCl at room temperature for 24 hours gives a soluble starch which may be precipitated from water solution by the addition of alcohol. In solution soluble starch is strongly dextrorotatory $(A)^{20}_{D}+202°$. It does not reduce Fehling's solution. Starch boiled for a short time with dilute (10%) HCl gives glucose. Maltose is an intermediate product. Various amounts of gummy substances, the dextrins are formed also.

XI. *Hydrolysis of Starch*

Acids may be used in the complete hydrolysis of starch in quantitative determinations, but in such determinations other substances than starch

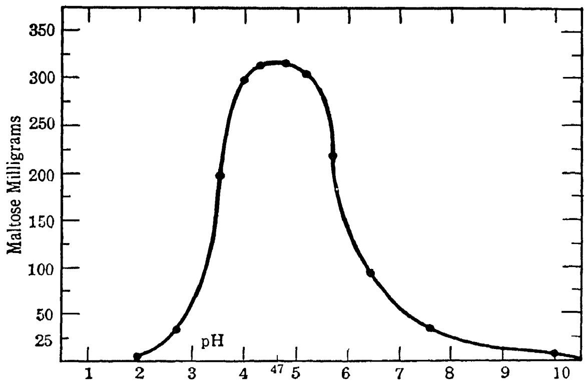


FIG. 31.—The relationship between the activity of wheat flour diastase and the pH of the medium. Temperature 27° C. Time 1 hr. Wheat flour 10 gms. (After Rumsey.)

may be hydrolyzed also, which introduces a great error in estimating starch. It is much better to hydrolyze the starch with diastase and then to filter off the insoluble material before complete hydrolysis to glucose by dilute acids. By this method starch only is determined.

XII. *Action of Diastase on Starch*

The cleavage of starch by diastase of malt or taka-diastase is a hydrolytic process. These diastase preparations should be considered as a group of substances rather than as single enzymes. The hydrolytic products of starch are glucose and maltose. Maltose is further converted into glucose if maltase is also present. Taka-diastase from *Aspergillus oryzæ* contains no maltase. The diastase reaction is quite complex. It involves

the cleavage of starch into a series of dextrins which give different colors with iodine. The ultimate change may be represented as follows:

$$\text{starch} \xrightarrow{\text{amylases}} \underset{(C_5H_{10}O_6)_x}{\text{dextrin}} \xrightarrow{\text{dextrinases}} \underset{C_{12}H_{22}O_{11}}{\text{maltose}} \xrightarrow{\text{maltase}} \text{glucose}$$

Irvine considers that the scissive products of starch are maltose 70% (72% theoretical) and glucose 30%. Two glucose molecules evidently are joined to form maltose, and the third glucose molecule is joined to these as in cellulose. The simplest possible starch molecule then contains at least three glucose molecules. Probably there are several of these units in most starches.

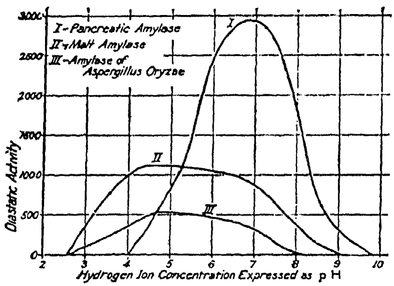


FIG. 32. - Influence of reaction upon the activity of three typical amylases. (After Shermal et al.)

The action of plant diastases is favored by moderate acidity, the highest activity being at pH 4-5. (Figs. 31, 32.) Increase of the temperature to about 65° C. greatly increases diastatic action, but the enzyme is rapidly inactivated at temperatures a few degrees higher. (Figs. 33, 34.) In germinating seeds there is a rapid formation of diastase during the period of rapid digestion of the stored starch. (Fig. 35.)

XIII. *Action of Bacteria on Starch*

The action of *Bacillus macerans* on 5% starch paste gives two dextrins (a and b) which are crystalline. Pringsheim and Langhans assign the following formula to these substances: a $(C_6H_{10}O_5)_4$ and b $(C_6H_{10}O_5)_6$. They obtained from the former a crystalline disaccharide $(C_6H_{10}O_5)_2$ and from the latter a crystalline trisaccharide $(C_6H_{10}O_5)_3$. All four of these substances have a sweet taste.

XIV. *Scissive Products of Starch*

The term *dextrin* is applied to substances which are polymeric with starch and formed from it by heating. Dextrins may occur as transitory products in plants when starch is acted upon by diastase. Certain dextrins may occur in a more permanent form as storage substances. The

sap of the epidermal cells of *Arum italicum* contains dextrins which turn reddish violet with iodine. The cell sap when evaporated gives a transparent sticky substance, colored violet with iodine solutions. Diastase action on starch gives a mixture of reducing sugars, 70% maltose, 30% glucose. When starch paste is left in contact with malt at 50° C., the mass rapidly liquefies and the solution becomes sweet owing to the production of maltose and glucose. The reducing power increases rapidly at first and continues to increase until the amount of maltose represents about 80% of the starch. In the digestion the blue-black coloration becomes less and less marked until various shades of red are obtained; finally the iodine gives no distinctive color. The constituents of the grains which are not hydrolyzed vary directly with the temperature, increasing considerably at the expense of maltose at a temperature of 60° C. This difficultly hydrolyzable part of the starch grain is composed of different constituents, some more readily converted into maltose than others. An almost theoretical yield of maltose can be obtained by the prolonged action of malt. The first stage of digestion consists in the digestion of amylose and requires about two hours for completion. In the second stage, amylopectin is hydrolyzed, a process which requires several days for completion.

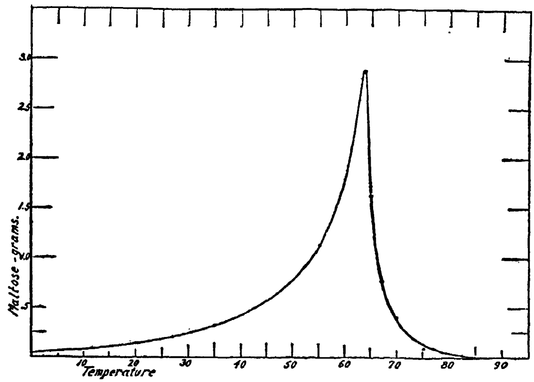


FIG. 33.—Relation of the temperature (° C.) to the activity of amylase. Autodigestion of wheat flour, time 1 hr. (After Rumsey.)

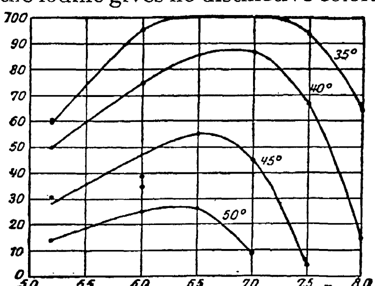


FIG. 34.—Influence of temperature and reaction upon the action of plant amylase. (After Sjoberg.)

Amylodextrin (4 hexose groups) was named by Nageli who first obtained it in crystalline form. It is the main constituent of soluble starch. It is a white powder slightly soluble in cold H_2O but readily soluble in hot water. It is strongly dextrorotatory($(\alpha)_D = +196°$). It does not

reduce Fehling's solution, but yields a blue color with iodine solution.

Erythrodextrin is a solid which dissolves readily in water, is strongly dextrorotatory ($(\alpha)_D = +196°$), and gives a red-brown color with iodine solution.

Achroödextrin is optically active ($(\alpha)_D = +192°$). It reduces Fehling's solution, but gives no color reaction with iodine. It has a sweetish taste.

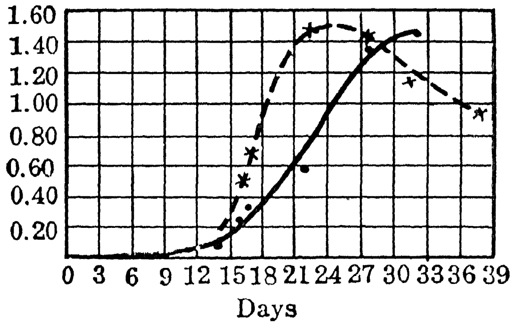


FIG. 35.—Course of formation of diastase in plants (*Phaseolus vulgaris*). ooo—cotyledons, xxx—leaves. (After Sjoberg.)

These dextrins as well as maltose were isolated by Lintner and Dull from products of malt action on starch by a long process of fractional precipitation with alcohol. Achroödextrin is not a single substance.

The dextrins are precipitated from aqueous solution by alcohol. Unlike starch, inulin, and glycogen, they do not give a precipitate with lead acetate. They are all dextrorotatory, and give a red color or no color with iodine. They do not reduce Fehling's solution when pure. They are converted into glucose on hydrolysis by mineral acids.

XV. *Starch Digestion in Germination*

A good picture of starch digestion can be obtained during the process of germination in cereals. In maize and wheat the endosperm contains large quantities of starch stored for the nutrition of the embryo. The layer of cells at the surface of the scutellar tissue produces diastase and excretes it into the endosperm. The secretion of the enzyme by this tissue may be easily demonstrated. Horning and Petrie picture the diastase as arising from mitochondria, which they show migrating in microscopic masses from the surface of the scutellar layer into the endosperm (Fig. 36). The cells next to the scutellum become depleted of starch coincident with the spread of the enzymes.

In the resting stage, the epithelial cells of the scutellum contain very finely granulated and semitransparent protoplasm. At the completion of the rest period the caryopsis will germinate if proper conditions are given for it. After germination has been started, it can be observed that the protoplasm of the epithelial cells becomes more granular. After 24 to 26 hours, digestion of the starch grains becomes evident and sugars appear in the cell sap. These soluble substances are absorbed by the scutellum. Part is used for growth of the scutellum and part is passed on to the embryo. The scutellum increases in size and its epithelium is thrown into glandular folds. The epithelial cells elongate and become swollen at the external end. They may break apart and grow into the endosperm, crushing the cells which have been depleted of starch. The picture of the excretion of mitochondria, their migration into the endosperm, and the digestion of starch, given by Horning and Petrie, is quite remarkable. A major difficulty is to account for the passage through the cell walls of such large bodies as mitochondria or even of molecules as complex as enzymes have been assumed to be. It would be much more easy to explain the effect of the scutellum on the digestion of starch in the epithelium if some soluble substance of small molecular size and quite diffusible through colloids such as ethylene should be produced by the metabolism of the scutellum and activate digestion in the endosperm cells.

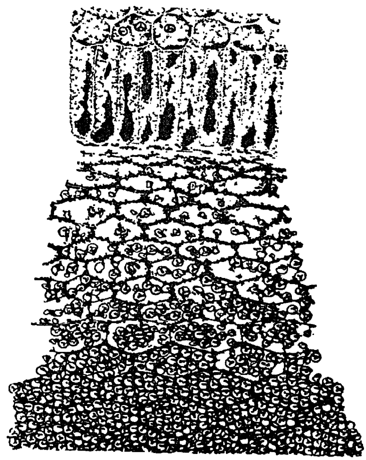


FIG. 36.—Digestion of starch in endosperm adjoining the scutellum in maize. (After Horning and Petrie.)

Many of the phenomena of enzyme actions indicate a rather high rate of diffusibility for enzymes into colloids, which would hardly be expected if complex molecules of great size were the diffusing substances. Probably there are diastatic enzymes present in the endosperm cells themselves which may be activated, for the endosperm cells themselves show starch digestion when the embryo is removed, although by no means as rapidly as when the embryo is present.

In the process of digestion the starch grain becomes etched characteristically. This is evidently due to the greater digestibility of certain parts of the grains. The etch figures of starch grains which are prominently

banded, such as potato or canna starch grains, show patterns which indicate that the granular parts of the concentric layers are more easily digested than the other portions.

XVI. *Glycogen* (8–9 hexose groups)

Glycogen is a common reserve in animals, but it is restricted in its distribution in plants to the fungi. It is especially abundant in *Saccharomyces cerevisiæ*, where it may form as much as 30% of the dry weight. It is also found in *Myxomycetes*, in flagellates, and in certain algæ, particularly the CYANOPHYCEÆ. The amount of glycogen present depends upon the physiological stage of development. In yeast it accumulates and then disappears, often rapidly. Glycogen appears in yeast cells in the early stages of fermentation as granules, later as small vacuoles, then finally in one large vacuole. Cells with large quantities of glycogen sink in Pasteur's solution. Budding is much more active in cells which have much glycogen. Glycogen is used up during great vegetative activity. It seems to be a temporary reserve in yeast. In the spores of *Mucor* it appears only after they have started to grow. In this case it is evidently an intermediate product. Even in yeast it may be an intermediate product formed for storage before it is used in alcohol formation. There is little doubt that the glycogens of plant and animal origin are identical.

Preparation. Glycogen may be prepared in a fairly pure state from yeast. The yeast should be well washed, ground with clean sand, and heated with two volumes of water. The filtrate is cooled and the glycogen is precipitated by strong alcohol. It may be further purified by reprecipitation from water acidified with acetic acid to remove proteins.

Properties. Glycogen is a snow-white amorphous solid, readily soluble in hot water, forming an opalescent solution. It can be precipitated again by alcohol if a small amount of salt is present. Glycogen solutions are strongly dextrorotatory ($(\alpha)_D = +189.9°$). Glycogen may be differentiated from starch by its red-brown coloration given with iodine. Glycogen does not reduce Fehling's solution. It may be hydrolyzed by mineral acids to glucose. Diastase hydrolyzes it to dextrin and maltose.

XVII. *Other Dextrosans*

Another dextrosan found in fungi is lichenin. Lichenin is found in lichens, particularly in Iceland moss. The lichenin is found in the fungus part of the lichen, not in the algal part. It yields dextrose on hydrolysis.

XVIII. *Levulosans*

a. Inulin is a levoratatory polysaccharide. It occurs in solution in the cell sap of a number of plants especially among the COMPOSITÆ, for

example, in the tubers of the dahlia, in artichokes, and in the fleshy roots of chicory. It is found in eight other families of flowering plants, and also in the alga *Neomeris*. Inulin is a storage form for the COMPOSITÆ, as starch is for some other plants. Inulin is often found present with starch in the monocots. Different species of the same genus of plants differ in their inulin content. *Scilla nutans* has inulin but no starch; *Scilla siberica* has both inulin and starch.

Characters. Pure inulin forms a white starchy tasteless powder with granules of a sphærocrystalline form. It swells and is readily dissolved in hot H_2O and in alkalis. It may be recovered from aqueous solution by the addition of alcohol or by freezing. Unlike starch, it does not give a paste with H_2O and does not give a blue color with iodine. Diastase has no effect upon it. Inulase is the enzyme concerned in its hydrolysis to d-levulose. Osmotic pressure measurements on inulin solutions indicate molecules of great weight, but the molecular structure of inulin seems less complex than that of starch.

Identification. Inulin occurs as sphærorcrystals in many plant sections after long treatment with strong alcohol. Sometimes it precipitates in the amorphous condition. In monocots this amorphous deposit often occurs. Inulin may be confused under the microscope with calcium phosphate, but it may be differentiated from phosphates by its solubility in hot water. Sections of material to be examined for inulin should be soaked for a long time in absolute alcohol. To precipitate and identify inulin, a saturated solution of orcin in strong alcohol is added, then hydrochloric acid is added and the tissue is boiled. The inulin masses disappear and a red color results from the reaction with orcin. Inulin gives the Molisch test for carbohydrates. Iodine gives to sphærocrystals of inulin a brownish coloration. A control reaction is necessary, for glycogen gives practically the same coloration. Basic lead acetate gives a white precipitate with both inulin and glycogen. Inulin gives only fructose on hydrolysis with mineral acids

Inulin-like substances occur in many monocotyledonous plants. Graminin occurs in grasses, for instance in *Festuca* and *Agrostis*. Irisin occurs in *Iris pseudacorus*. Phlein is the levulosan of timothy, *Phleum pratense*, and is found also in *Phalaris arundinacea*. Sinistrin is found in *Scilla maritima*. Triticin commonly occurs in *Triticum repens*. All of these levulosans have the same empirical formula $(C_6H_{10}O_5+H_2O)_n$. They are all levorotatory and give only levulose on hydrolysis. They are fairly soluble in cold water, and are usually hard to crystallize. They may bear probably the same relation to inulin that dextrins bear to starch.

XIX. *Interconversion of Carbohydrates*

The conversion of sugars into starch and vice versa are easily effected in plants. Starch may appear as a photosynthetic product five minutes after the exposure of leaves to light. The reconversion of starch to glucose is also rapid. The conditions which determine which process, synthetic or analytic, shall be most active is probably determined by enzymes and the mass action of the products of photosynthesis. When the photosynthetic rate is high, the piling up of soluble sugars causes the synthetic

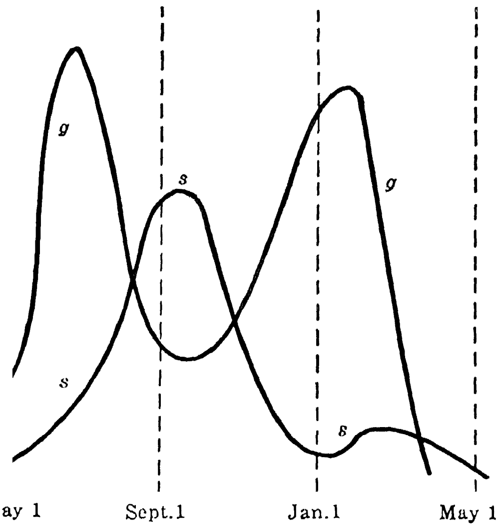


FIG. 57.—Curves representing the variations in sucrose (s) and of (g) in onion bulb. (After Leclerc du Sablon.)

phase to be predominant in the leaf cells. When the soluble sugar concentration decreases through transport or by utilization, the amyloclastic enzymes effect the digestion of starch.

The balance between reducing sugars, sucrose, and starch is evidently determined by the temperature to which the plant is exposed. Low temperatures, near the freezing-point, favor the conversion of starch into glucose and sucrose. Thus during the autumn the sugar content of plant parts may be increased at the expense of stored starch. This leads to a greater freezing-point depression, and the plant is not so easily frozen; also, it may be undercooled to a greater degree than when it is in the summer condition. The production of two molecules of monosaccharide

from a disaccharide doubles the freezing-point depression. In onion bulb (Fig. 37) there is commonly such a transformation of sucrose into glucose during the colder months of the year. When the temperature again rises in spring there is a re-formation of the disaccharide. In *Colchicum* corm (Fig. 38) the temperature is a factor of major importance in determining the equilibrium concentrations of starch and sugar. Starch is converted into soluble sugars in autumn, and these reach a maximum during the winter. In spring, before photosynthesis occurs, there is a reconversion of sugar into starch. The starch concentration becomes highest and the sugar concentration nearly reaches zero during the periods of most rapid photosynthesis and of highest temperature in summer.

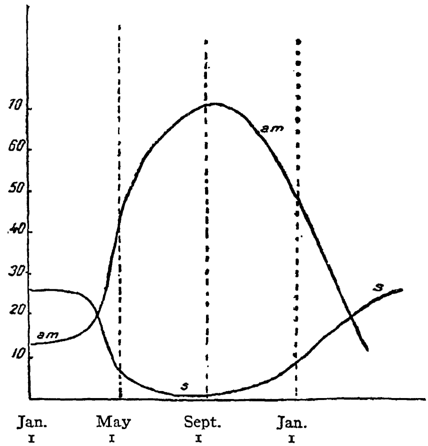


FIG. 38.—Curves representing the variations in starch (am) and of sugar (s) in *Colchicum* corm.

The conditions which determine the place of deposit of reserve carbohydrates have not been elucidated. Evidently starch deposit is associated with the presence of leucoplasts, so the presence or absence of these organs in the cells of certain tissues may determine the points of starch deposit. In the Brassicas, such as kohlrabi, turnips, Brussels sprouts, and cabbages, the place of deposit of reserve carbohydrates is limited locally. Hybrids of these species show great variation in the points of starch deposit. Evidently heredity is of importance in determining the localization of reserve deposits.

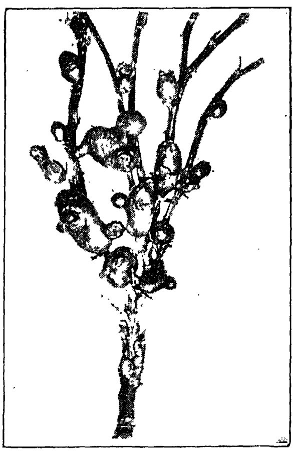


FIG. 39.—Green aerial tubercles of potato grafted on tomato.

After injury, interference with conduction in the phloem leads to the deposit of starch above the wound. In grafts of potato on tomato there may be produced aerial tubers on the potato scion (Fig. 39). The form of deposited carbo-

hydrate, whether inulin or starch, is determined by the depositing cells in the stock although photosynthesis may have occurred in a scion which normally deposits starch.

The roots of trees in cold climates generally are exposed to less extremes of temperature than the trunk or limbs; consequently there are

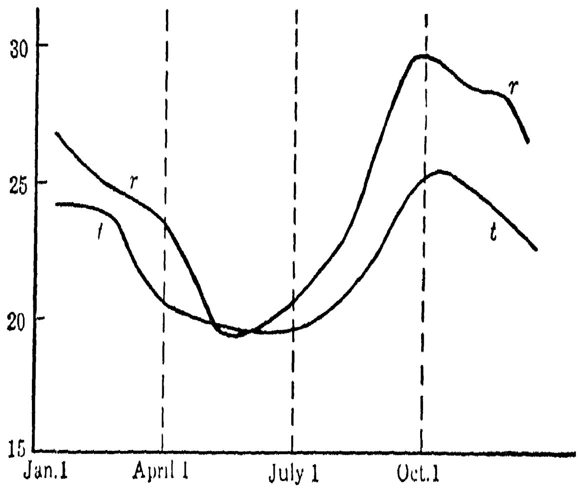


FIG. 40.—Curves representing the variations in reserve carbohydrates in the root (r) and the stem (t) of oak.

differences in the balance between the forms of carbohydrates in roots and limbs at various periods of the year. This may lead to deposit or withdrawal of the carbohydrate reserves in different parts of the plant. The reserve carbohydrates of the oak (Fig. 40) are drawn from the root during the period of spring growth more than from the stem. During the summer, deposit in the root is more rapid than in the trunk.

CHAPTER XI

NATURAL GUMS

The naturally existing gums were formerly thought to be carbohydrates of the general formula $(C_6H_{10}O_5)_n$. The researches of O'Sullivan have shown they are not simply carbohydrates but that they are of glucosidal nature. They give on hydrolysis sugars mixed with complex acids of high molecular weight.

The natural gums are more or less transparent amorphous substances. Some dissolve completely in H_2O. Others swell up in water to form gels. All of the gums are insoluble in alcohol. Alcohol precipitation may be used as a crude means for the estimation of gums. The gums are levorotatory whereas the dextrins are dextrorotatory. Basic lead acetate precipitates them from colloidal solution, and this reagent is commonly used in analysis to remove gums during sugar determinations. Gums yield on hydrolysis chiefly galactose and pentoses such as arabinose and xylose. Generally gums are not very easily hydrolyzed. Eighteen to twenty-four hours boiling with dilute HCl is required for complete hydrolysis. Since the gums contain galactose, their oxidation with HNO_3 gives chiefly mucic acid, but some saccharic and oxalic acids are also formed. Gums occur in nature as salts of potassium, calcium, or magnesium, combined with the organic acid radical.

A crude classification of the gums is based on their solubility in water. They may be divided into gums such as arabin, which are completely soluble in water; gums which are partially soluble in water, for example cerasin and bassorin; and mucilages and pectic bodies that merely swell up in water to form a jelly.

I. *Formation and Properties of Gums*

Arabic. Gum arabic forms a colloidal solution in H_2O. The nucleus is an acid, arabic acid of the formula $C_{23}H_{38}O_{22}$. Ten per cent H_2SO_4 changes arabic acid to meta-arabic acid which swells up in water but does not dissolve.

Tragacanth. Thus gum occurs in species of *Astragalus*. It is exuded in ribbons through cracks in the cortex. Only about 8–10% of gum tragacanth is soluble. This portion represents mainly salts of Ca, Mg, and K. Sixty to seventy per cent of gum tragacanth represents insoluble salts which only swell up to H_2O to a gel. The soluble portion of tragacanth is

said to contain a polyaraban trigalactan, geddic acid. Geddic acid is an isomer of arabic acid. The part of the gum that is insoluble in H_2O is a combination of pentoses, tragacanthose, and xylose, with bassoric acid ($C_{14}H_{20}O_{13}$).

Gummosis. Wound gum does not swell in H_2O as much as gum tragacanth. It is insoluble in dilute (10%) H_2SO_4 and NaOH. On wounding trees, particularly peach or cherry trees, there are frequently pathological changes in the tissues which cause the production of gums. Frequently such gums are produced by the action of bacteria. In such cases the gum may swell up and block the lumens of conducting tissues, a condition frequently found in the diseases known as *wilts*. Gummosis is usually associated with the action of pathogens in the tissue.

II. *Mucilages*

Complex carbohydrate substances that form slimy liquids with H_2O are commonly referred to as mucilages. The outside walls of many plants such as *Spirogyra* have a coating of mucilage. Mucilage is probably produced from the hydrolysis of wall substances in the algæ. Mucilage frequently exudes from the secreting hairs on the surface of higher plants. It is found sometimes in the canals or ducts of plant stems. Here, also, it is probably produced by the hydrolysis of cell walls. Some mucilages yield mainly mannose, for example tulip mucilage; others, such as the mucilage of Irish moss, *Gigartina sp.*, yield galactose; but more generally the mucilages are galactan-araban compounds.

The function of mucilages on epidermal surfaces seems to be to reduce transpiration, as in epiphytic plants such as the orchids. They may favor water absorption by aërial parts. Also, they may reduce diffusion through the walls, and in some cases prevent the penetration of ions of heavy metals.

The presence of mucilage on the surface of leaves favors the undercooling so that they may not freeze so easily. Mucilages covering the surfaces of seeds, as in flax, enable the seed to imbibe water and thus hold a moisture supply for the germinating embryo. In certain water plants the mucilages on the surface of seeds certainly prevent too great desiccation of the embryo. In some seed coats, such as the mustard seed, the special mucilage cells are of importance in the imbibition of the moisture requisite for germination.

The mucilages appear to be produced by the partial oxidation of cell wall constituents either as a normal process or under the action of bacteria.

Since the mucilages are usable carbohydrates in many plants, they must be regarded as possible reserve forms, although this function is probably minor.

CHAPTER XII

PECTIC SUBSTANCES

Pectic substances are common in succulent fruits, pears, apples, gooseberries, currants, etc. They are found also in fleshy roots, such as carrots, turnips, and beets. Their chemical character is not perfectly known. Pectic substances on complete hydrolysis give galactose, arabinose, galacturonic acid, and other substances. According to Frémy the hardness of unripe fruit is due to a substance called pectose (protopectin) deposited in cell walls, which is ultimately converted into pectin.

The discovery of pectin in plant juices by Braconnot in 1833 was followed by extensive researches upon it. The net results of the work of these earlier investigators were to establish that pectin bodies are carbohydrate derivatives possessing acid properties, to show the presence of arabinose, carboxyl, and mucic acid yielding groups in the pectin complex, and to name and describe a long list of pectin bodies. The individuality of many of these substances in different plants may well be doubted.

According to Ehrlich, pectin is a derivative of arabino-galacto-tetra-galacturonic acid containing four COOH groups, three of which are esterified with CH_3OH, while the fourth forms a salt with Ca or Mg in the plant to form insoluble materials.

Before Ehrlich, Von Fellenberg had shown the presence of methyl groups in ester combination with pectic acid. Ehrlich's important contribution was the identification of the substance to which pectin owes its acid properties. This substance is galacturonic acid, an isomer of glucuronic acid and a half-way oxidation product between galactose and mucic acid, represented by the formula $C_6H_{10}O_7$ or $COH.(CHOH)_4.COOH$. Ehrlich was the first investigator actually to isolate galactose from pectin, although its presence had been inferred previously from the fact that pectin yields mucic acid on oxidation. Calcium and magnesium were also found by Ehrlich in the pectin complex.

Von Fellenberg regards pectin as essentially the methyl ester of pectic acid, since the methoxy groups are split off by saponification with sodium hydroxide. Not all pectins are fully methoxylated, however; part of the methoxyl groups may be replaced by hydrogen or metals. Other groups may also be substituted for methoxyl. Tutin considers that there is a partial replacement of the methoxyl groups by isopropenyl groups

in apple pectin, and Sucharipa has submitted evidence that in the "albedo," or white inner rind of lemons, methoxyl groups are replaced by cellulose units. This would account for the formation of pectocelluloses.

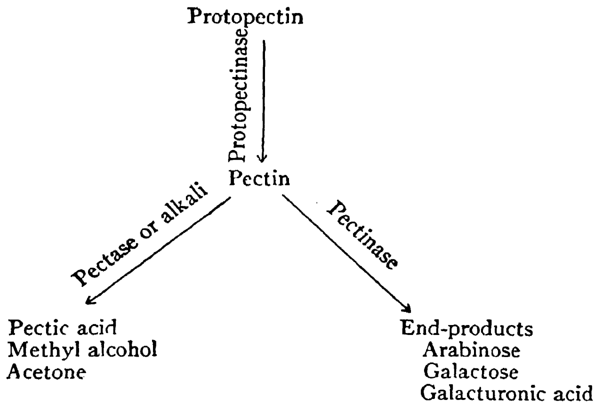
Ehrlich describes the naturally occurring pectin of plants as "the calcium-magnesium salt of a complex anhydro-arabino-galactose-methoxyl-tetragalacturonic acid," but regards the form of combination as uncertain, except that the arabinose units in the complex are very loosely bound while the union of the galactose is unusually strong. Pectic acid is the product remaining after hydrolyzing out the araban and then splitting off the calcium and magnesium by treatment with dilute acids. The product still contains the methoxyl groups and is spoken of as an "ester acid," but is regarded by Ehrlich as essentially a galactose-galacturonic acid, that is, an equimolecular union of galactose and galacturonic acid, analogous to the paired glucuronic acids that occur in the animal body.

No serious conflict exists between the views of Von Fellenberg and Ehrlich. The "ester acid" described by Ehrlich shows the same chemical behavior as Von Fellenberg's partially methoxylated ester or pectic acid.

Pectin substances then may be defined as "derivatives of galacturonic acid." This view is consistent with the time-honored recognition of pectin bodies as carbohydrates possessing acid properties. Methoxyl groups occur in ester combination with galacturonic acid; other groups also enter into the pectin complex, but their method of linkage to the galacturonic groups is not yet known. According to Tutin, pectin is the dimethyl-isopropenyl ester of pectic acid. Pectin is commonly found in the middle lamella of the cell walls of plants. It is extractable with alkalies and certain salts. The Ca or Mg salt of this substance as it exists in the plant is known as *protopectin*, or variously named *pectose* or *pectinogen*. Pectin upon the loss of the CH_3O groups goes over into pectic acid. Fellenberg thinks one OCH_3 group is lost less easily than the others, and some preparations of pectic acid may yet contain one such OCH_3 group. When pectin loses these methoxy groups, it loses its ability to gel. Protopectin in the cell wall may be hydrolyzed by protopectinase to produce pectin.

Pectin may be hydrolyzed by pectase or by alkali with the formation of pectic acid, methyl alcohol, and acetone. On hydrolysis with pectinase, pectin yields the end-products arabinose, galactose, and galacturonic acid. Pectase seems to act on the ester linkages while the other enzymes do not.

The relationships between these enzymes and their substrates may be represented as follows:



The acidity of the medium exerts different effects upon the three enzymes as shown in Figs. 41 and 42.

Of three forms of pectic substances which occur in nature, protopectin is found in succulent root vegetables and in unripe fruits, soluble pectin generally in ripe fruits, and pectic acid in certain unripe acid fruits and in rotten vegetable tissues generally. The real existence of protopectin has been questioned by Tutin, who finds that all the pectin of apples may be extracted by boiling with water, provided that the material is sufficiently finely divided to break up all protective cell walls. On the other hand, Sucharipa has shown that finely divided lemon "albedo," after thorough extraction of the soluble pectin, still contains other pectin bodies which are not soluble until after hydrolysis. They appear to be combined with cellulose as "pectocelluloses." While Tutin's experiments prove that the pectin of apples is all in the soluble form, it appears from Sucharipa's work that protopectin, or the insoluble form, exists in some of the more permanent vegetable tissues.

Some insight has been obtained by Von Fellenberg on the probable mechanism of the formation of fruit jellies. When fruit juices containing pectin are boiled with sugar and organic acids under proper conditions, a viscous solution is obtained which sets to a jelly on cooling. Prolonged boiling in the presence of organic acids, however, destroys the jelly-making power of the pectin, probably because of the formation of pectic acid. According to Von Fellenberg and to Sucharipa, jelly does not form when fully methoxylated pectin is boiled with sugar alone. If organic acids, such as malic or tartaric, or their calcium or magnesium salts, are added to the pectin-sugar solution, jelly formation usually occurs. On the other hand, it has been found impossible to prepare a jelly from a mixture of

pectic acid and sugar, either with or without acids. These findings indicate that jelly formation is accomplished by neither fully methoxylated pectin nor pectic acid, but by partially methoxylated intermediates. To produce a jelly, the substance must be soluble in the sugar solution and give a sufficient rigidity to satisfy the well-known physical requirements of a good jelly. The fully methoxylated pectins fail because their solutions are too fluid. Pectic acid fails because it is not soluble in the sugar solution and therefore yields a non-cohering suspension of gelatinous particles, distributed unevenly through the syrup, instead of a uniform solid mass.

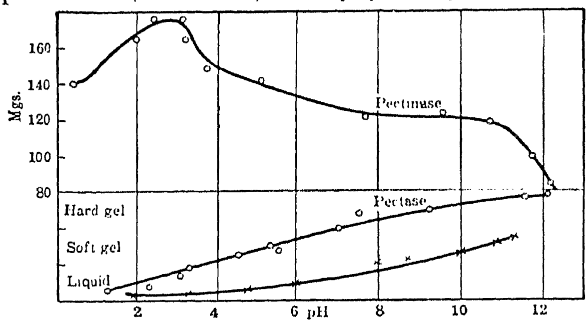


FIG. 41.—Relation between hydrogen ion concentration and activity of pectinase and pectase; crosses show controls. (After Davison.)

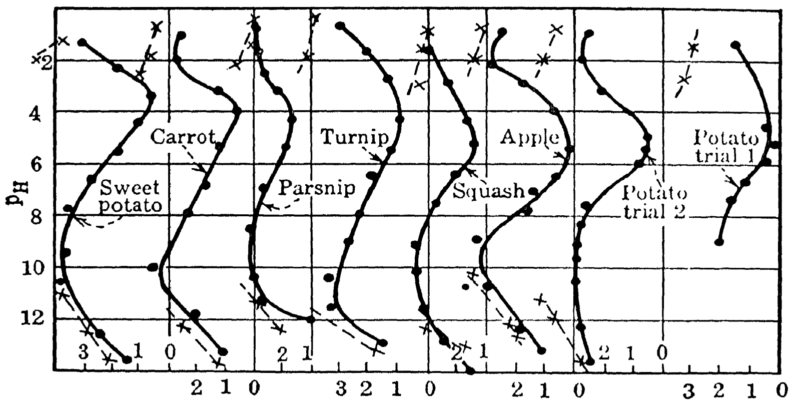


FIG. 42.—Rate of maceration of various tissues by protopectinase at various hydrogen ion concentrations; dotted lines show controls. (After Davison.)

The formation of a firm gel from pectic acid which may be present in the middle lamella is accomplished by soaking the tissue in lime or alum water, as in the manufacture of pickles or watermelon preserve. There is an effect also of the calcium in making a firm gel of other cell-wall colloids.

CHAPTER XIII

CELL-WALL CONSTITUENTS

The higher plants contain cellulose as the characteristic wall-forming material. When the cells are young, the cellulose seems to be almost free from admixture with other substances. But when the cells grow older, they generally show the presence of many other substances in the wall. Probably differences in the chemical behavior and staining reactions of cell walls are due to the presence of these substances other than cellulose. But it seems probable that cellulose itself should not be considered as a single chemical unit, but as a group of substances. It may be that the principal differences between the celluloses of different plants is merely in the degree of hydration.

I. *Cell-Wall Formation*

On the division of the cell nucleus into two daughter nuclei it may be observed frequently that the linin fibers that stretch between each pair of chromosomes after division form knots of material at the division line between the daughter nuclei. These swellings may be formed by the pulling together of the fibers themselves at first, but later it is evident that much additional material is piling up there. The division line or cell plate is at first made up of calcium pectate almost entirely. Later the deposit on each side of the pectate layer is of almost pure cellulose. The cause of the deposition may be difficult to find, but one should always remember that substances which lower the surface tension tend to accumulate at the surface, and this principle should hold for the interface between protoplasts. From the phenomena of wall formation in *Pythium* and in some zoöspores it would seem probable that the accumulation at the surface is caused by physical forces, and that frequently cell walls are precipitation membranes, at least at the start. The banding of cell walls in many cases indicates a periodicity in the deposit. This banding is related to the growth in cotton fibers, being periodic with the day and night. The periodic precipitation of substances infiltrated into the original wall may be concerned also in this banding. The banding of the walls of woody fibers and sclerenchymatous cells may be due to infiltration and periodic precipitation of substances in the colloidal wall in the same manner as in the Leisegang periodic precipitation in other colloids. There seem to be differences in composition of the layers of such banded walls.

The question as to whether the infiltring substances are in combination with the cellulose of the wall in ester linkage or whether they are merely adsorbed has not been settled. The cell wall contains a very great number of substances with widely varying chemical properties. Many times the physical properties of the cell wall are determined more by the infiltrated substance than by the cellulose itself. The wall constituents, in fact, are frequently classified on the basis of the substances which are found present with the cellulose. The substances called lignocelluloses, pectocelluloses, and cutocelluloses are so named because it is supposed that lignin, pectin, and cutin are combined with the cellulose in ester-like linkages.

II. *Cellulose*

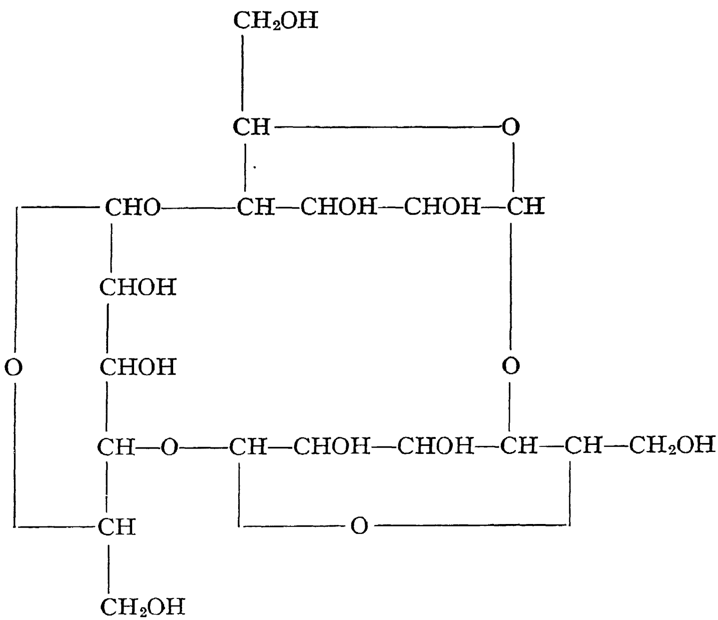
Cellulose is found abundantly in plants as almost a pure substance. Cotton fibers contain when air dry 91% of pure cellulose, 8% of water, and 1% of impurities, comprising wax, oil, pectic substances, and mineral matter. Pure cellulose is insoluble in all ordinary solvents. However, it is soluble in a solution of zinc chloride, and also in an ammoniacal solution of cupric oxide. From these solutions the cellulose may again be obtained apparently in its original form. Cellulose can be partly hydrolyzed by alkalies or acids, but for complete hydrolysis concentrated acid or alkali is required. If cellulose is partly hydrolyzed until the fibers are swollen in acid or alkali and the further action stopped, there is produced a substance which gives a blue color reaction with IKI solution. This is the hydrocellulose reaction. Hydrocellulose or closely related substances are frequently found in plants. The product of complete hydrolysis is glucose only. Cellulose forms esters with organic and inorganic acids. On account of their use in the industries, the cellulose di-, tri-, tetra-, penta-, and hexanitrates are of importance, as are also the acetates. Strong oxidizing agents such as chromic acid, potassium chlorate in strong HCl, and nitric acid convert cellulose into a series of oxidation products known as *oxycelluloses*. The oxidation products differ according to their method of formation. They are characterized by the fact that they yield a relatively large quantity of furfurol on boiling with HCl, much as the pentosans do. Oxycelluloses seem to be present in the fibers of cereal straws.

In the plant, cellulose is mainly a structural material, not entering into the metabolic reactions and not serving as a reserve substance generally. However, cellulose is digested in the process of vessel formation in all vascular plants, and many fungi possess the ability to digest cellulose for their nutrition. No considerable difficulty is offered to the protoplast in softening or dissolving its surrounding cellulose wall, and

this occurs frequently in cell fusions. Evidently a cellulose dissolving enzyme, cellulase, is quite commonly found in cells, and probably the cellulose of the wall is more drawn on as a reserve substance than has been stated usually in texts of plant physiology.

Cellulase may be prepared from certain wood-rotting fungi, such as *Xylaria*, by water extraction. It may be precipitated by the addition of alcohol. The presence of cellulase in such fungi enables them to penetrate the host tissue and also yields a supply of carbohydrate available for their metabolism.

The size of the cellulose molecule and its structural arrangement can hardly be said to be determined. The empirical formula $(C_6H_{10}O_5)_n$ expresses its composition. The value of n is claimed to be anything from 1 to 34. It is generally agreed, however, that the molecule is more complex than starch.



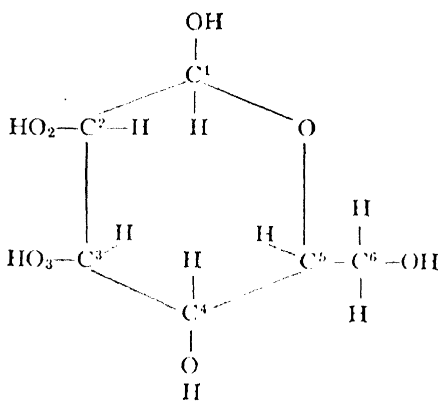
Anhydrotrisaccharide formula for cotton and flax cellulose.

From X-ray spectrophotometric measurements of the space lattice of cellulose, Sponsler and Dore came to the conclusion that the glucose molecules composing cellulose were arranged in parallel chains which run

lengthwise of the cellulose fiber in the cell wall. The unit of cellulose structure they consider to be two glucose molecules joined together in glucosidal linkage by primary valences. These units occupy the space of a parallelopiped with sides $6.1 \times 5.4 \times 10.25$ Ångström units. The atoms are so arranged in this space that a number of planes occur whose spacings bear simple numerical relations to the longer dimension of the parallelopiped 10.25 Å. That is, there are sets of planes in the space lattice of the atoms so that the interplanar spacings are $\frac{1}{2}$, $\frac{1}{3}$, or $\frac{1}{4}$ of the longitudinal dimension 10.25 Å; consequently there are planes with spacings 5.15, 3.40, and 2.58 Å apart.

The units of cellulose are arranged in parallel chains running continuously through the ramie fiber. The chains are spaced rectangularly 6.1 and 5.4 Å. apart. In the lateral direction the chains of cellulose units are held together by secondary valences. After methylation cellulose yields on hydrolysis 2, 3, 6 trimethyl glucose, for only the 2, 3, 6 carbons have replaceable hydroxyls. The 1, 4, 5 carbon atoms must be joined so as to make their hydroxyls unreactive. There are no aldehyde reactions shown by cellulose; consequently there are no straight chain aldehyde structures in the molecule.

The normal stable form of glucose shows an amylene oxide ring as follows:

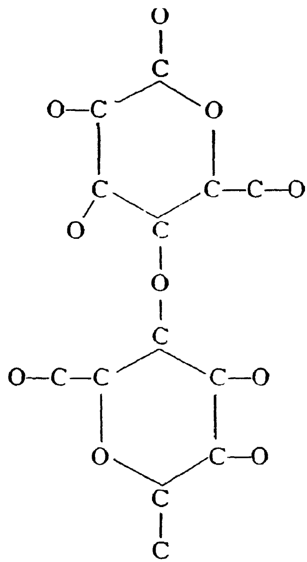


-d-glucose amylene oxide ring.

There is found also a β-d-glucose with the amylene oxide ring structure. In this form the OH's on the first and second carbon atoms are on opposite sides of the ring.

Cellulose is built up of two glucose units with the amylene oxide

ring structure, the two molecules being joined through the 1–1 and 4–4 carbons as follows:



The units are arranged with a diagonal distance of 3.98 Å. between the spacings in the tangential position around the cylindrical wall of the cellulose fiber. In the tangential as well as in the radial direction the units are held together by secondary valences. In the longitudinal direction primary valences are involved. This idea is given support by the tensile strength of the fibers. Further evidence of the differences in the nature of the linkages in the three directions is given by the termal expansion data of wood. The coefficient of expansion of wood in the direction of the grain and of the wood fibers is only one-tenth of the coefficient of expansion in a direction at right angles to the grain. The thermal expansion is associated with molecular vibration, and this should be greatest where the freedom of movement is greatest, that is where the cellulose units are held together by secondary valence forces (Fig. 43). The swelling of wood fibers in water can be explained in a similar manner. There is no swelling longitudinally, but much swelling in the lateral direction of the wood where the molecules are held together by the secondary valences alone and allow easy penetration of water molecules. The water molecules are held by the secondary valences of the oxygen atoms arranged in the

lateral direction. Each oxygen may take up one H—OH. In the swollen fibers there is a different spacing in the space lattice than in dry fibers. The increase in the lateral spacing due to the introduction of water molecules on the secondary valences of the oxygen amounts to 2% of the diagonal dimension. Yet there is no increase in the longitudian dimension.

Cellulose esters, such as the methyl esters, may be made by the replacement of the OH groups on the second, third, and sixth carbon atoms of the glucose unit. The fibrous structure may still be maintained after esterification because all that is necessary is to open out the fibers radially to accommodate the replacing groups.

In cellulose derived from wood the glucose is associated with xylose units. In the oxidation of cellulose to oxycellulose the primary alcohol

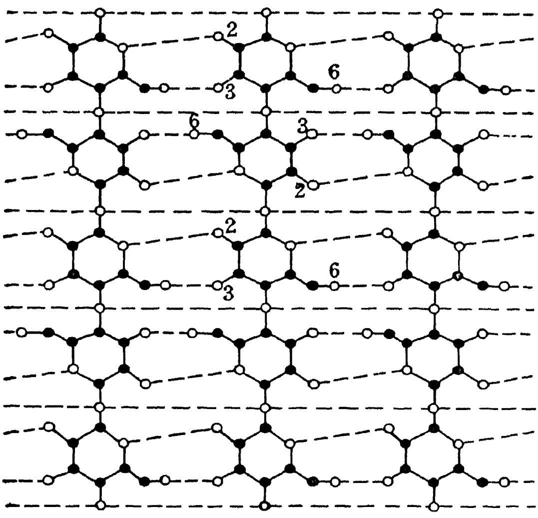


FIG. 43.—Tangential section through a ramie fiber showing three chains of glucose units. Dark lines indicate primary valence bonds; broken lines indicate probable general direction of secondary valence forces. (Sponsler.)

group on the sixth carbon atom becomes oxidized first to CHO and then to COOH, producing glucuronic acid. Glucuronic acid then may undergo decarboxylation to form xylose. The xylose so formed will have the amylene oxide structure. Consequently, there will be no disturbance of the original cellulose structure in the fiber. In some cases it seems that all of the glucose units in a chain will be oxidized to xylose, forming then

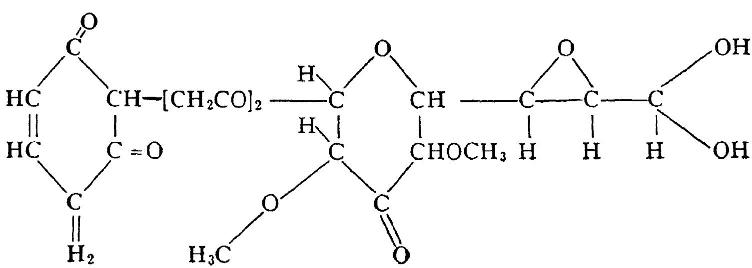
a xylan chain. We may then have xylan and cellulose units alternating. This oxidation seems to occur more frequently at the exposed surfaces of the cylindrical tube on the outside and the inside of the fiber.

III. *Lignin*

Most important of the cellulose compounds in the cell wall is lignocellulose, or lignin, which is the principal constituent of woody fibers. De Candolle gave this substance the name *lignin* (Latin *lignum*, wood). On account of the microchemical reactions with orcin and phloroglucin by which it can be easily differentiated from cellulose, the distribution of this substance has been much studied in plants. When tissue containing lignin is heated with a 1% solution of phloroglucinol in alcoholic solution and then a drop of hydrochloric acid is added, there is produced a red coloration. Some tissues possess compounds which may yield phloroglucinol, and for the production of the reaction in these tissues the addition of hot HCl is all that is required. Spruce wood consists of about 50% pure cellulose, 16% other carbohydrates, 30% lignocellulose (lignin), and 4% of other substances.

Lignin has generally been considered to consist of two major groupings, an aromatic nucleus of great complexity joined to a carbohydrate nucleus either of cellulose or cellulose derivatives. In the manufacture of white paper from wood-pulp it is desirable to remove the aromatic constituent from the wood fibers because the tissue containing lignin quickly turns brown on oxidation, especially in sunlight. The lignin is extracted as completely as possible by digestion with "sulphite liquor" which contains sodium sulphite. The composition of a lignocellulose may be represented as follows: a resistant cellulose-α, 65%; less resistant cellulose-β which yielded furfural, 15% (total cellulose percentage of dry weight, 80%); lignone, 20%.

Based on its chemical reactions the following formula for lignone has been proposed:



The percentages of the lignone fraction in various lignocelluloses differ, as do also the other constituents of the wood fibers of various plants. It is probably for this reason that there are various chemical and physical reactions shown by different wood fibers.

Lignin appears to be produced by the condensation of coniferyl and hydroxyconiferyl alcohols which probably are produced within the cell wall while it is undergoing lignification. Coniferyl alcohol may be produced from tannic aldehyde. Through this series of condensations, lignin formation seems to be bound up with a very common class of plant substances, the tannins.

PART III

PART III

FATS, LIPIDES, AND WAXES

CHAPTER XIV

FATS

I. *Classification of Fats*

The fatty substances of plants may be divided into three classes, the true fats, the phosphatides, and the waxes. These substances differ in their physiological properties and in their functions in the cell.

The true fats are esters of the trihydroxy alcohol glycerin with various fatty acids. The phosphatides are esters of glycerin with fatty acids much the same as the true fats, but they have one fatty acid replaced by phosphoric acid and to this is joined a base which may be either choline or amino-ethyl alcohol. The waxes are esters of monohydroxy alcohols.

Those fats which serve principally as storage forms for energy are to be considered as nutrient lipoids. The storage fats are mainly glycerides of fatty acids formed *in situ* in the cell from carbohydrates or protein. They are not soluble in the vacuolar sap, and consequently cannot be translocated as fat but must first undergo hydrolysis to glycerin, which is quite diffusible, and fatty acids, which are somewhat diffusible. The fatty acids probably undergo further cleavage before undergoing translocation to great distances.

The fats may accumulate in quantity in globules, making up a third or more of the dry weight of fatty seeds. They may also appear in a finely colloidal condition in the protoplasmic emulsion, so finely dispersed that they cannot be separated from other constituents by centrifugation. In this condition they cause the protoplasm to be deeply stained black by osmic acid, which is a common microchemical reagent for fats. When in the form of globules, the fats can be stained by Sudan III or Scharlach R, and their location in the cell can be determined by these stains. The state of division of the fat is of much importance in determining its usability in metabolic processes since the globules themselves form a different phase from the cell sap.

The fats of plants differ greatly in the fatty acids which are combined with glycerin. The fatty acids commonly found belong to seven series, classified on the basis of the unsaturated bonds in the carbon chain.

The fully saturated or acetic acid series consists of acids of the general formula $C_nH_{2n}O_2$. The oleic acid series consists of acids with the general formula $C_nH_{2n-2}O_2$, and which contain one double bond in the carbon chain. The members of the linoleic series have the formula $C_nH_{2n-4}O_2$, the linolenic series $C_nH_{2n-6}O_2$, the clupandonic series $C_nH_{2n-8}O_2$. Acids of the last series occur in animals but are not of common occurrence in plants. There is a series of fatty acids occurring in plants which is unsaturated and partly oxidized, the ricinoleic acid series $C_nH_{2n-2}O_3$. The unsaturated oxyacids of the ricinoleic acid series have the general formula $C_nH_{2n-2}O_3$. The most common member of this series is ricinoleic acid, the 18-carbon acid of castor-beans, whose formula is probably as follows: $CH_3—(CH_2)_5—CHOH—CH_2—CH=CH—(CH_2)_7—COOH$. There are several isomerides of this acid. All of the known members of this series have 18 carbon chains. The carbon chain of fats is almost always unbranched. This may be taken as indicating an origin from stright chain compounds, or from short chain units which combine to form straight chains.

In all of these series of saturated or unsaturated acids the carbon chain nearly always contains an even number of carbon atoms, that is, the value of n is nearly always even. Acids containing an odd number of carbon atoms are found in plants, but they do not exist commonly as fats but are found usually as esters of monohydric alcohols. Plants seem commonly to synthesize fats from acids only with an even number of carbon atoms in the chain. This indicates that the C atoms are introduced into the chain in pairs or in even numbered multiples. Fatty acids with uneven numbers of carbon atoms in the chain have been synthesized artificially.

Tiglic acid, $C_5H_8O_2$, in the oil of *Croton tiglium*, is an exception to the usual configuration of the fatty acids since in addition to having an odd number of carbons it also has a branched chain. This is, however, an acid of low molecular weight. There are no exceptions when only acids of high molecular weight are considered.

There seems to be a preference for the synthesis in plants of 18 carbon chain acids, especially in the oleic, linoleic, linolenic, and ricinoleic series. The C_{18} acids in all of these series are by far the most abundant, but saturated fatty acids of shorter chain length are found in plants. In the saturated series the C_{18} chain, stearic acid, is abundant.

The fatty acids combined with glycerin in the molecule of fat may be all of one kind, or two of one kind and one of another, or all of different kinds; however, there seems to be a preference for forming fats with all the hydroxyl groups of the glycerin combined with a single kind of acid to form such compounds as glyceryl tripalmitate or glyceryl trioleate.

Where a number of fatty acids are found in a tissue it is probably more proper to consider them as constituents of mixed fats rather than as being fatty acids mixed in the molecule, unless it is proven that there are different fatty acids in the molecule. This mixing of the acids which are esterified with a single glyceryl radical has been shown to be true, however, in a number of cases. The members of the fatty acids of the saturated series which occur in plants are as follows:

$H\ COOH$	or CH_2O_2	formic acid
CH_3COOH	or $C_2H_4O_2$	acetic acid
C_3H_7COOH	or	butyric acid
$CH_3(CH_2)_4COOH$		caproic acid—found in cocoanut and palm-nut oils, also in butter
$CH_3(CH_2)_6COOH$	or $C_8H_{16}O_2$	caprylic acid
$CH_3(CH_2)_8COOH$	or $C_{10}H_{20}O_2$	capric acid
$CH_3(CH_2)_{10}COOH$	or $C_{12}H_{24}O_2$	lauric acid—laurel oil
$CH_3(CH_2)_{12}COOH$	or $C_{14}H_{28}O_2$	myristic acid—nutmeg butter
$CH_3(CH_2)_{14}COOH$	or $C_{16}H_{32}O_2$	palmitic acid—most plant fats
$CH_3(CH_2)_{16}COOH$	or $C_{18}H_{36}O_2$	stearic acid—most plant fats
$CH_3(CH_2)_{18}COOH$	or $C_{20}H_{40}O_2$	arachidic acid—peanut oil
$CH_3(CH_2)_{20}COOH$	or $C_{22}H_{44}O_2$	behenic acid—oil of benne
$CH_3(CH_2)_{22}COOH$	or $C_{24}H_{48}O_2$	lignoceric acid—peanut oil

Acids of this series with ten carbon atoms or less form glyceryl esters which are fluid at 30° C. The consistency of the fats becomes harder on going to longer carbon chains. The stearates are quite hard at room temperatures. The carbon chains contain no asymmetrical carbon atoms so there are no optical isomers and the pure fats do not show a rotation of the plane of polarized light. In those fats with acids of this series which do rotate the plane of polarized light, the rotation is probably due to the admixture of lecithin, phytosterol, or other optically active substances.

$C_{16}H_{30}O_2$	hypogæic acid from peanut oil (*Arachis hypogæa*)
$C_{18}H_{34}O_2$	oleic acid from olive-oil (*Olea europæa*)
$C_{22}H_{42}O_2$	erucic acid from mustard-seed oil (*Brassica* and *Sinapis sp.*)
$C_{22}H_{42}O_2$	brassidic acid from mustard-seed oil

Oleic acid is by far the most abundant of the members of this series.

All acids of this series have one double bond or two unsaturated carbon atoms in the carbon chain. The position of this double bond in the chain

may be various, so that the structural isomers may have different properties owing to the differences in position of the double bond.

For instance, three acids are known as follows:

$$\begin{aligned}
&1.\ CH_2=CH\text{—}CH_2\text{—}CH_2\text{—}COOH\\
&2.\ CH_3\text{—}CH=CH\text{—}CH_2\text{—}COOH\\
&3.\ CH_3\text{—}CH_2\text{—}CH=CH\text{—}COOH
\end{aligned}$$

When boiled with alkali a shifting in position of the double bond may occur, and this may also be brought about in the organism. The double bond makes possible cis- and trans-isomers, with arrangements as shown in the formula, of which a number of cases occur, as in hypogæic acid and gaidic acid $C_{16}H_{30}O_2$; erucic and brassidic acids $C_{22}H_{42}O_2$. The arrangements of the groups in the cis-trans isomers are as follows:

$$\begin{array}{ccc}
\begin{array}{l} R_2\text{—}C\text{—}H \\ \quad\ \ \| \\ R_1\ \ C\text{—}H \end{array} & \text{and} & \begin{array}{l} R_2\text{—}C\text{—}H \\ \quad\ \ \| \\ H\ \ \ C\text{—}R_1 \end{array}
\end{array}$$

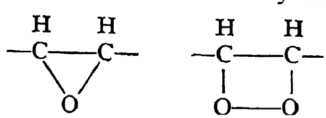
When such groupings are split at the double bond, they yield identical substances.

In oleic acid the double bond is probably in the exact middle of the chain. A migration in position to between the α and β carbons may occur on treatment with alkali. Oleic acid gives stearic acid on reduction, by taking up two hydrogens at the double bond. An oleic acid with the double bond between the sixth and seventh carbon atoms from the unoxidized end has been shown to occur in plants. Erucic acid has the double bond between the eleventh and twelfth carbon atoms from the unoxidized end. Brassidic acid is the cis-trans isomeride of erucic acid.

On reduction in the presence of nickel salts, two hydrogens may be introduced at the double bond in all acids of the oleic series, thus producing a saturated acid. This process is used commercially in producing solid fats from cotton-seed oil and other oils, for use in cookery.

The larger the number of unsaturated bonds that occur in a fat, the more easily it is oxidized and the more unstable it is. There seems to be some conversion in plants of saturated acids to unsaturated acids for rapid metabolism. The saturated acids are to be regarded as storage forms while the unsaturated acids are more easily metabolized.

Oxygen may be added to unsaturated fatty acids to form peroxide-like structures. Either one or two atoms may be introduced as follows:



On oxidation in the presence of water, two hydroxyl groups can be introduced at the double bond of acids of the oleic series. On further oxidation the chain usually breaks at the double bond, producing fatty acids of shorter carbon chains which are saturated. The length of the two carbon chains so produced will depend upon the position of the double bond.

The acids of the linoleic series commonly found in plants all have the formula $C_{18}H_{32}O_2$. A number of isomers of this formula are possible owing to differences in position of the two double bonds. In elæostearic acid which occurs in Japanese wood oil it has been established that the double bonds are between the fifth and sixth and ninth and tenth carbon atoms. Owing to their unsaturation, the oils of this series are very easily oxidized, and form peroxide-like structures with the addition of oxygen.

The acids of the linolenic series are very easily oxidized. Fats containing them are known to be good drying oils. The drying is due to oxidation, with the formation of solid waxy substances. Certain metallic salts such as those of zinc, copper, lead, barium, and calcium hasten the oxidation, and for this reason they are used as "driers" in paints. Oxidation can be hastened also by partly oxidizing the oil, since the oxidation process is autocatalytic. The commercial practice is to boil the oil in kettles open to the air, until a mixture of partly oxidized fat is obtained which will dry quickly on exposure to the air in thin layers, as in painting.

Acids of the unsaturated series seem to be more reactive than acids of the saturated series; they tend to form glycerides more quickly and are more easily oxidized or metabolized by plants than acids of the saturated series.

II. *Temperature Relations of Fats*

The character of the fat is largely dependent upon the nature of the fatty acid present in it. In the saturated fatty acid series there is a rise of about 20° C. in the melting-point of the acid with the addition of each carbon atom, owing to the increase of the molecular weight. These differences show up in the fats also. The melting-point is much used in determining the properties of fats. The melting-point of the fat determines at what temperatures it will be in the fluid state, and this is of great importance in cell physiology. Fats containing fatty acids of the unsaturated series are fluid at lower temperatures than those containing acids of the saturated series. A good comparison can be made between the liquid glyceryl trioleate of olive-oil and the glyceryl tristearate of fats which are hard at ordinary temperatures, such as cacao-butter.

Plants in their internal temperatures follow closely the temperature

of their surroundings; they are said to be *heterothermous* or *poikilothermous* organisms. This is also true of many lower animal organisms. But, in contrast, the higher animals have reached their state of development and their ability to extend over broad latitudes largely owing to their maintaining a fairly constant temperature independent of the temperature of their surroundings. They may be referred to as *homothermous* or *homoiothermous* organisms; they regulate their internal temperatures.

The condition of the fat, whether liquid or solid, is very important for determining its mobility in the cell and probably in determining the mobility of the protoplasm also, since the fats are distributed in the colloidal condition throughout the protoplast as well as in globules of microscopic size. The rate of movement of protoplasts is slowed down remarkably by lowering the temperature. This can be nicely demonstrated by lowering the temperature of *Chara* cells on a controlled temperature stage of the microscope and observing the rate of movement of the chloroplasts. The fluid condition of fats is important in making them more easily broken into finer particles for easy digestion by the enzymes of the protoplast. The finer the state of division is, the more surface there is exposed to the action of lipases or other enzymes concerned in fat metabolism.

It would seem then of a distinct advantage for plants to have their fats in a fluid condition, particularly in those plants which are adapted to growth in northern regions. The fats of most plants are liquid at the average temperatures of the temperate zones, 15° to 20° C. Yet there are many plants in cold climates whose fats are still liquid at –30° C. This is shown particularly by the conifers, in which the leaves are held over winter in a more or less functioning condition and in which oil is a common storage form in winter. The fats of many tropical plants are of a much higher melting-point than those of northern origin. A comparison of the melting-points of the fats of some plants of temperate climates with those of tropical origin may illustrate this point (Table 14), although a hard and fast generalization of this kind cannot be made.

TABLE 14

Plants of temperate climates	*Melting-point of fat*
Pinus picea	27° — 30° C.
White pine oil (*Pinus strobus*)	18° — 20° C.
Brassica nigra	17.5° C.
Flaxseed oil (*Linum usitatissimum*)	27° C.
White mustard oil (*Sinapis alba*)	16.3° C.

TABLE 14—*Continued*

Tropical plants	*Melting-point of fat*
Croton-oil (*Croton tiglium*)	7° C.
Cocoanut oil (*Cocos nucifera*)	16° — 23° C.
Nutmeg oil (*Myristica fragrans*)	39° — 44° C.
Cotton-seed oil (*Gossypium herbaceum*)	3° — 4° C.
Cacao-butter (*Theobroma cacao*)	23° — 27° C.
Peanut oil (*Arachis hypogæa*)	2° — 3° C.

The differences shown here are largely due to differences in acids of the saturated and unsaturated series rather than to differences in the molecular weight of the fats.

> "*And as oil is an excellent preservative against the injuries of cold, so it is found to abound in the sap of the more northern trees; and it is this which in evergreens keeps their leaves from falling.*" *Stephen Hales, Vegetable Staticks, p. 322.*

The degree of unsaturation of fatty acids is determined by the temperature at which the plants grow. Plants grown farthest from the equator have the most unsaturated fats. Also those grown at high altitudes have a high iodine number (Table 15).

TABLE 15

IODINE NUMBER OF OIL FROM PLANTS GROWN AT VARIOUS LATITUDES [1]

		Linum usitatissimum	*Helianthus annuus*	*Cannabis sativa*
Archangel	64° 30′ N. Lat.	195–204		
Leningrad	59° 44′ N. Lat.	185–190		163.7
Omsk	55° 50′ N. Lat.		140.4	
Moscow	55° 50′ N. Lat.	178–182	135	
Voronesch	51° 40′ N. Lat.	170	125–128	
Schatilowo	53° 00′ N. Lat.	174		158.5
Kuban-Odessa	45° 46′ N. Lat.	164	120	155.5
Taschkent	41° 26′ N. Lat.	154–158		
Aschabad	37° 30′ N. Lat.		117	

The presence of oil in the colloidal condition in protoplasts favors undercooling of the tissues and for this reason the production of oil by plants in winter may have a function of protection from injurious low temperatures. Especially would this seem to be the case if the oil produced tended to prevent the solidification of the lecithin or other con-

[1] After S. Ivanow, *Die Klimaten des Erdballs und die chemische Tatigkeit der Pflanzen.*

stituents of the protoplast. There is good evidence that lecithin is concerned with the regulation of permeability, so that changes in properties of the lecithin-containing parts of the protoplast by admixture with oils of low melting-point would tend to maintain a fluid condition of the protoplast and in this way maintain semipermeability of the cell.

There are many plants living in regions with low temperatures in winter which have the habit of forming oil from carbohydrates during the fall and winter. Of trees, the birches, basswood, pines, and plum are often referred to as "fat trees" since they transform most of their starch into oil during the winter. Most of the conifers have a similar tendency. In contrast to this group, the "sugar trees" or "starch trees" form but little fat, soluble carbohydrates and starch being the predominant storage substances in winter. To this latter group belong the oaks, apple, pear, elms, hazel, and the ash trees. A good comparison of the low temperature resistance of these groups can be made by comparison of the northern limits of the common plum and pear varieties. However, other factors than this one difference in the production of liquid fat are concerned in the ability of the plants to withstand low temperatures.

III. *Energy Value of Fats*

Fats are efficient forms for the storage of energy. They represent the highest caloric value per unit of weight of all the storage substances of cells. Fats are always the preferred storage forms in animals, in which group there is need of economy in weight on account of their movements, in contrast to the non-motile plant. In plant sperms and eggs and the motile forms of plants the storage of fat is more common than the storage of carbohydrates.

The average values for the heat of combustion in large or kilogram calories per gram of dry weight of fat, protein, and carbohydrate show clearly the advantages of fats as forms for the storage of energy.

	Large calories per gram
Fats	9.3
Protein	5.71
Carbohydrate	4.1

IV. *Hydrolysis of Fats—Saponification*

Fats can be split on hydrolysis either with alkali, or with steam under pressure, or by lipases, into glycerin and fatty acids. The reaction proceeds according to the following equation:

$$
\begin{array}{lcc}
CH_2OOC_{17}H_{35} & & CH_2OH \\
| & & | \\
CH\ OOC_{17}H_{35} + 3H_2O \rightarrow & 3C_{17}H_{35}COOH + & CHOH \\
| & & | \\
CH_2OOC_{17}H_{35} & & CH_2OH \\
\text{glyceryl tristearate} & \text{stearic acid} & \text{glycerine}
\end{array}
$$

If alkali, such as potassium hydroxide, is used in this process a potassium salt of stearic acid is produced, which is a potassium soap. The process of hydrolysis by alkali is called *saponification*, for a soap is made thereby. The soaps of alkali metals are soluble in water but little soluble in oils. The soaps of alkaline earth metals are not soluble in water but more soluble in oils. Soaps are formed in the protoplast, and the balance between the soaps of univalent cations such as sodium and divalent cations such as calcium is of great importance to the protoplast. A proper balance between these soaps must be maintained in the protoplast to maintain normal permeability. If the calcium soaps predominate, the cell is not sufficiently permeable to water phases or substances soluble in water. If the sodium soaps are too abundant, the cell is not permeable to fat-soluble substances. A proper balance between these ions is maintained by proper conditions of mineral nutrition.

The formation of soaps by reaction with alkali can be used to estimate the average molecular weight of the acids contained in fats. A weighed quantity of fat is boiled under a reflux condenser with a known quantity of standard alkali, which should be more than sufficient to combine with the fatty acids which it is expected will be formed. The excess of alkali is titrated, and from the quantity of alkali used to combine with the fatty acids the saponification number or Koettstorfer number is obtained. This number is expressed as the number of milligrams of potassium hydroxide consumed in the complete saponification of one gram of fat. The value of the saponification number lies in determining the average molecular weight of the acids in the fat, since for acids of low molecular weight one gram of fat will contain more acid molecules than for acids of high molecular weight. Determinations of the saponification number of fats in germinating seeds indicate that in sprouting the fatty acids of the seed are broken up into acids of shorter carbon chains with smaller molecular weight.

V. *Lipases*

The lipases, the enzymes concerned in the hydrolysis of fats, are of common occurrence in plants. Lipases are easily demonstrated in germinating seeds in which oil is the storage material. The lipases are not specific in their activity; any lipase preparation will hydrolyze any fat. In fact, they are more to be regarded as esterases since they will hasten the hy-

drolysis of a large number of esters other than those of glycerin. Some lipases show a higher sensitivity to the presence of certain fatty acids so that glycerides of these acids are more rapidly hydrolyzed than glycerides of other fatty acids. The action of the lipases is favored by acidity, the optimum of acid being variously stated as between n/60 and n/100. Possibly the solubility of the acid in the fat globule may in part determine the effect of acidity on hydrolysis. The actual acidity or pH is determined usually in the water phase. The acidity of the oil phase may be different from this. It is known that the presence of fatty acids which are soluble in fats favors the action of lipases as in germinating seeds. The action of lipase is not arrested by 12% acetic acid. The temperature optimum for lipase is from 23° to 43° C., but the activity is not destroyed by temperatures much higher than this. Lipase is more thermostable than most enzymes.

The lipases have been shown to have a synthetic as well as an analytic action on esters. Ethyl butyrate can be synthesized from butyric acid and ethyl alcohol in the presence of lipase.

VI. *Preparation of Lipase*

Lipase may be prepared by grinding castor-beans or other oil seeds with water until all of the cell walls are broken. The resulting emulsion of oil, protein, etc., is allowed to ferment at 24° C. A scum of oil containing the ferment rises to the surface, and this may be separated from the aqueous layer.

VII. *Rancidification of Fats*

The lipases favor the oxidation and rancidification of fats since they cause hydrolysis of the glycerides. The free fatty acids are easily oxidized by atmospheric oxygen in the presence of moisture. This is particularly true of the unsaturated acids. The less unsaturated fatty acids the fat contains, the less it will tend to be oxidized or to become rancid. In the process of becoming rancid there is first the appearance of oxyacids, owing to the oxidation of unsaturated acids in the fats, then cleavage may occur in the partly oxidized acids, yielding acids of short carbon chains whose presence favors the further hydrolysis of the fat. The glycerol liberated on hydrolysis is easily oxidized, and it rapidly decreases in amount. If lipases are present with the fat, as in olive-oil, the production of free acids favors their action so that the process becomes autocatalytic.

VIII. *Chemical Tests of Fats*

Another method of estimating the number of acids of high and of low molecular weight in fats is the determination of the proportion of the

fatty acids produced upon hydrolysis which are volatile in steam distillation. The acids of low molecular weight can be distilled in a current of steam, and the titration of the distillate will give a measure of the acids of low molecular weight contained in the fat. The Reichert-Meissl number is the number of cubic centimeters of n/10 alkali required to neutralize the soluble volatile acids obtained from five grams of a hydrolyzed fat by steam distillation. This method is of value in determining differences in plant fats, particularly with regard to those constituents which are often responsible for flavors.

The unsaturated fatty acids have the property of forming addition compounds with the halogens, two atoms of iodine, bromine, etc., being introduced into the fat at each double bond. The quantity of iodine so combined may be used to determine the number of double bonds present in the acids of the fat. The iodine number, or Hübl number, is the number of grams of iodine absorbed by 100 grams of the fat. Determinations of the iodine number on the fats of germinating seeds show that in the early stages of this process the unsaturated acids disappear, attendant upon an increase in the total number of acids present. This indicates that oxidation of the unsaturated acids occurs at the double bonds with subsequent cleavage and formation of saturated acids of shorter carbon chains.

The quantity of free acid contained in a fat is of value in indicating the nature of physiological changes in these storage forms. If there is hydrolysis of the fat, the free acids produced will be absorbed and dissolved by the fat, since they are soluble in the fat phase. The number of milligrams of potassium hydroxide required to neutralize one gram of fat then is a measure of the free acids present in the fat. This value is called the acid number. The production of acids which go into solution in the fat favors the action of lipases and also favors auto-oxidation and hydrolysis. Rancid fats have a high acid number. The acid number of fats increases during the germination of seeds, indicating that hydrolysis of the fat is taking place, making possible the translocation of fatty reserves of the seed.

The number of hydroxyl groups contained in the fatty acids is of importance in determining the properties of the fat. The hydroxy acid content can be estimated by acetylation of the fat by boiling it under a reflux condenser with acetic anhydride, then driving off the excess acetic acid by boiling with water, and then determining the amount of acetic acid which is bound by the hydroxyl groups. An increase in the number of hydroxyl groups of the fatty acid is an indication that oxidation of the fat is occurring. This type of oxidation releases energy, and there is no reason why energy liberated by such transformations of fats is not usable

by the cell. Fats are quite commonly transformed to carbohydrates in germination, and probably such oxidations as these are preliminary to the transformation. Castor-beans contain some of these partly oxidized acids.

IX. *Synthesis of Fats*

The storage fats of animals have been proved to be formed from the fats of the ingested plant material, and the same fats found in the food appear in the adipose tissues of animals. Different fats of plants will produce differences in the fats of the animals to which they are fed. Cotton-seed oil is a good example of a fat that will affect the body fats and the butter fat of animals to which it is fed. Animals also can synthesize fats from ingested carbohydrate material, and it is in animals that the changes involved in this transformation have been studied most. There is very conclusive evidence that the same series of transformations occurs in plants. The polysaccharides are first hydrolyzed to monosaccharides. Then by a complex series of reactions these sugars are transformed into fats.

The ordinary mechanism of fat synthesis from carbohydrates can be considered to occur in three steps, (1) the formation of glycerol, (2) the synthesis of the fatty acid, and (3) the esterification of the glycerol and fatty acids. According to Ivanow the unsaturated fatty acids are probably formed after the appearance of the saturated fatty acids.

1. GLYCEROL FORMATION

Glycerol is quite easily formed from carbohydrates, in fact, its aldehyde, glyceric aldehyde, is often considered as an intermediate product in the synthesis of carbohydrates. The glyceric aldehyde produced by the cleavage of glucose at the center of the chain is easily reduced to glycerol, and this seems to be a very common process in plants.

2. FORMATION OF FATTY ACIDS

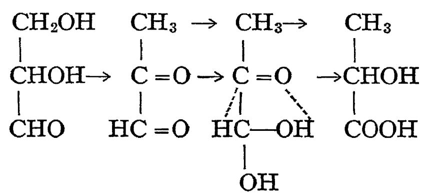
In the formation of the fatty acids from hexoses two types of reaction must occur, (1) reduction of the hydroxyl groups, (2) condensation in order to form the long carbon chains of the fatty acids. There are some group reactions of the carbohydrates which indicate the probable manner in which these changes are brought about. In carbohydrate metabolism it is a common reaction that an alcoholic hydroxyl is reduced with a transfer of its oxygen to another carbon atom. This is a simultaneous intramolecular oxidation and reduction. This reaction probably is the best explanation of the formation of lactic acid from sugar, a very com-

mon change in the breaking down of sugar. Probably the first step in the formation of lactic acid from hexoses is a disjointing of the carbohydrate chain between the β and γ carbon atoms. This cleavage is common in secondary alcohol groups in other compounds,

$$CH_2OH—CHOH—CHO/H—CHOH—CHOH—CHO$$

and produces two molecules of glyceric aldehyde from one molecule of glucose. This may be reduced to glycerol or further transformed.

The next step in the production of glycerol is the reduction of the alcoholic hydroxyl group, and the oxidation of the aldehyde end of the molecule, transforming the glyceric aldehyde into lactic acid. This is a common reaction for compounds of this nature.



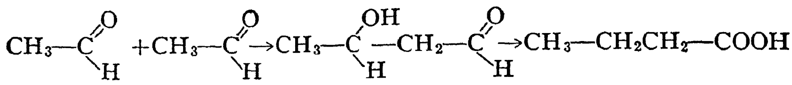
Not much energy is liberated by the first cleavage reaction, but the second oxidation reduction rearrangement involves the liberation of considerable energy, in fact, this reaction is the chief source of energy for many bacteria that ferment hexoses to lactic acid.

A common reaction of the hydroxy acids such as lactic acid is to break down into the lower aldehyde, hydrogen, and carbon dioxide. The hydrogen so produced may be nascent and available for the reduction of glyceric aldehyde to glycerol. All of the processes up to this point in the process might be considered to occur instantaneously with merely a shifting of atoms in the molecule.

The next stage involves the splitting off of one molecule of CO_2 which evolves considerable energy.

$$CH_3CHOH\ COOH \rightarrow CH_3CHO + CO_2 + 2H$$

Lactic acid by this process produces acetaldehyde, CH_3CHO. Aldehydes have a tendency to polymerize with little or no energy change. Acetaldehyde on polymerization gives aldol, a four carbon chain which by the Cannizzaro reaction, a simultaneous oxidation and reduction reaction, produces butyric acid.



acetaldehyde aldol butyric acid

The oxidation and reduction occurring here is the same as the reaction in the formation of lactic acid from glyceric aldehyde; the reduction of the alcoholic hydroxyl is accompanied by the oxidation of the aldehyde to a carboxyl group. There has been formed in this series of reactions butyric acid, a member of the saturated series of fatty acids. Probably other acids of this series are formed by a similar series of condensations involving acetaldehyde, a two carbon chain, because in plants these acids always contain an even number of carbon atoms.

Acetaldehyde might be a product coming directly from the photosynthetic reaction in chlorophyl-bearing cells, but most of the evidence indicates that the photosynthate seems to go through the carbohydrate stage, with fat formation as a secondary process in the green leaf as elsewhere in the plant.

Many bacteria seem to use this series of reactions regularly for producing butyric acid, CO_2, and H_2 from hexoses. Lactic acid itself may be used in the production of butyric acid, thus indicating that it may be a regular intermediate product in the butyric acid synthesis by bacteria. In the process of fermentation of glucose by bacteria normal caproic acid, $C_6H_{12}O_2$, and also caprylic acid, $C_8H_{18}O_2$, are produced simultaneously with butyric acid. This indicates that higher members of the series may be produced by the same series of reactions, three or four molecules of acetaldehyde entering into the chain.

Emil Fischer explained the origin of the long carbon chain, particularly the formation of the C_{18} series of acids by the polymerization of trioses or hexoses with a reduction of most of the carbons of the chain and oxidation of the end carbon. Stearic, oleic, linoleic, or linolenic acids might originate from three hexoses or six trioses. According to Fischer, pentoses might also take part in the formation of other chains such as palmitic acid, $C_{16}H_{32}O_2$, from two pentoses and one hexose molecule. If this be true, it would be difficult to see why C_{11} and C_{17} acids should not be formed also, but these are not commonly produced. All of this seems to point to the probability that this series of reactions suggested by Fischer may not be followed in plants in the production of fatty acids. Most of the evidence seems to point to the formation of fatty acids from a two carbon chain unit such as acetaldehyde.

The rather rapid conversion of starch to sugar and then to fat in pine-needles, in plum buds, and in other plant parts during the period of de-

creasing temperature in autumn indicates that the main transformation is from carbohydrate to fat. In ripening seeds the increase in fat content seems parallel to the decrease in carbohydrate. Two tables (16 and 17) given by Leclerc du Sablon will illustrate this:

TABLE 16

WALNUT

Date of gathering	*Per cent fat*	*Per cent glucose*	*Per cent sucrose*
6 July	3	7.6	0
1 August	16	2.4	0.5
15 August	42	0	0.6
1 September	59	0	0.8
4 October	62	0	1.6

TABLE 17

ALMOND

Date of gathering	*Per cent fat*	*Per cent glucose*	*Per cent sucrose*	*Per cent starch and dextrins*
9 June	2	6	6.7	21.6
4 July	10	4.2	4.9	14.1
1 August	37	0	2.8	6.2
1 September	44	0	2.6	5.4
4 October	46	0	2.5	5.3

In animals there is good evidence that fats may be produced from proteins. There is no reason for assuming that similar processes do not occur in plants, but the evidence is in favor of carbohydrates being the principal constituents of the cell which are transformed into fats.

Priestley says that fat metabolism probably starts from carbohydrates and takes place in light, but that given a liberal supply of carbohydrates it may take place in the dark. He also calls attention to the fact that apparently fat synthesis can only take place in the cell that is going to use the fat or store it, for there are no fatty substances in the transpiration stream.

3. ESTERIFICATION OF GLYCEROL AND FATTY ACIDS

The next step in fat formation is the esterification of the glycerol and fatty acids. When mixed, fatty acids and glycerol will remain unchanged

for years without esterification. A catalyst or an enzyme is necessary to make the esterification occur at a rate sufficient to account for fat formation in plants.

Probably the same lipases concerned in the hydrolysis of fats may bring about their synthesis, or different enzymes may be involved in each process. Still Ivanow has shown that seeds have the catalysts necessary for both the synthesis and hydrolysis of fats.

Ivanow used poppy seeds which are normally rich in fats containing saturated fatty acids, and flaxseeds which are rich in fats containing unsaturated fatty acids. The lipases of each kind of seeds were extracted with glycerol. To the glycerol extract of dry poppy seeds free oleic acid was added. In three months the acid number fell from 44.6 to 34.84. When oleic acid was added to a glycerol extract of dry flaxseed, the acid number fell from 80.04 to 51.4 in 3 months and 16 days. There was no decrease in the acid number of checks which had been boiled to stop the action of enzymes. Evidently there is esterification of the fatty acid and glycerin by substances in the seed, by catalysts or enzymes which are soluble in glycerin.

When the glycerol extracts of lipases were made from seed containing 60–80% of water, a water content which would normally occur in germinating seed, the reaction was reversed. The glycerol extract of poppy seed containing 60–80% water with oleic acid showed an increase of acid number from 14.4 to 23.8 in 2 days. In flax the acid value rose in 16 days from 16.4 to 24. The increase in free fatty acid must have been from fat dissolved in the glycerol extract obtained from the seed.

The percentage of water in the seed seems to determine whether synthesis or hydrolysis predominates. There should be synthesis under the conditions of desiccation which prevail in the ripening of seeds, and hydrolysis under conditions yielding sufficient moisture for germination.

X. *Fat Deposits in Elaioplasts*

The synthesis of fats occurs in the carbon-assimilating cells of algæ such as *Vaucheria*, and it seems to be the reserve form preferred over starch in many monocotyledonous plants. In fact, the fat might be formed directly from short chain aldehydes formed during photosynthesis. Quite frequently oil deposition in globules occurs in or around particular cell structures called *elaioplasts*. These bodies are quite highly developed in some cells of monocotyledonous plants. They are colorless bodies rich in protein constituents; they resemble leucoplasts. They are easily found in *Vanilla sp.*, *Ornithogalum*, *Psilotum*, and the corolla hairs of *Gaillardia* flowers. These elaioplasts are of various shapes and are fre-

quently associated with the nucleus. In some cases they seem to be formed by the aggregation and subsequent breaking down of chloroplasts which give rise to oil in the process of their degeneration. Fat droplets are frequently found closely associated with the chloroplasts of many green algæ.

XI. *Fats in Fungi*

The spores of algæ and fungi are usually rich in fats. The sclerotia of many fungi have abundant fat; in ergot of rye, *Claviceps purpurea*, fats may represent 60% of the dry weight. Fat globules may be demonstrated in the mycelium of many filamentous fungi. Free fatty acids are usually abundant in the fats of these fungi. The ability of many fungi and bacteria to resist toxic agents is in many cases associated with the high content of waxy substances containing sterols and phosphatides. The tuberculosis bacillus (*B. tuberculosis*) shows this relation very well.

XII. *Storage of Fats*

In the whole plant kingdom a great variety of storage relations of the fats are found. There may be fat only as the storage form, or fat and starch together as in the grasses, or fat and reserve hemicelluloses, or celluloses mainly as in nutmeg and in many nuts. The conifers have great quantities of storage fat with some glucose in the seed. In all of these seeds there are, of course, the cellulose of the wall materials and other carbohydrates of the seed coats, and proteins, which make up part of the dry weight. The percentage of fats varies greatly in seeds, but it is often higher in those seeds in which storage in parts of the embryo is predominant over storage in the endosperm. Thus in the wheat grain (*Triticum vulgare*), fats represent 1.8% of the dry weight. Most of this fat is found in the embryo. In flax (*Linum usitatissimum*) fat represents 33.6% of the dry weight. Most of the fat of flax is contained in the cotyledons of the embryo. A similar comparison can be made between corn (*Zea mays*) and castor-bean seeds (*Ricinus communis*). The cocoanut contains 67% of fat, which is present in the endosperm along with hemicellulose.

There is some storage of fats in the stems of plants and in tubers, underground root stalks, corms, and bulbs. In many of the underground parts of plants, carbohydrates are favored over fat as the storage forms. There are, however, many exceptions to this generalization, for instance the chufa (*Cyperus esculentus*), whose tubers have a high fat content.

The latex of the plants of the family EUPHORBIACEÆ and of other families contains considerable quantities of fat globules in emulsion. The transport of fat as such is possible in lactiferous tubes.

XIII. *Carbohydrate-Fat Transformations*

In the "fat trees" the transformation of starch into fat begins in the autumn from about September to November, depending somewhat upon the temperature. The quantity of oil reaches a maximum during January or February, and then there is a reversal of the reactions. Carbohydrate is re-formed from the oils during the early spring months. Basswood twigs may contain 9–10% of fat during the winter, and the bark of the walnut may contain as high as 50% of fat. The same cycle of changes can be brought about by low temperatures produced artificially and at different times of the year, so that the transformation is not merely a periodic phenomenon but is dependent upon the temperature. During winter the transformation of carbohydrates to fats in the leaves of pines growing in the colder regions is quite remarkable.

In seeds there is often a transformation of carbohydrate to fat during the process of ripening. In the development of flaxseed the testa is first formed, followed by the development of the embryo. The testa is rich in carbohydrates, largely pentosans whose function is protection of the seed and which take no part in later nutrition. The fats, synthesized *in situ* in the cells of the embryo, are formed from glucose with a disappearance of sucrose and starch. Proteins play a minor part in the fat synthesis if they are used at all. Glucose is first used up, then the sucrose and starch of the cells are hydrolyzed and converted into fat. There is an intense oil formation for about two weeks during the middle of the seed development period (Fig. 44). The carbohydrates of the stem are mobilized by transformation to glucose, and move into the embryo as a falling gradient of concentration is established in that direction through the formation of oil as an independent phase in the cells. The acids of the fats first formed

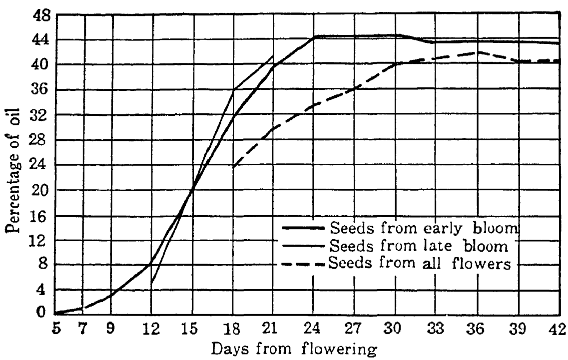


FIG. 44.—Percentage of oil in flaxseed from the early full bloom (flowers tagged July 2), from the late bloom (flowers tagged July 12), and from bulk seeds (all flowers). The first bulk sample was obtained at St. Paul, Minn., in 1927 by pulling the plants on July 14, about 18 days after the first flowers appeared. (Dillman.)

are saturated; later in the development of the seed unsaturated acids are formed in greater quantity, and these tend to make the oil more fluid.

XIV. *Fat Utilization in Germination*

In the cotyledons of the peanut there is an increase of dry weight owing to the fixation of oxygen in the process of transforming fat into carbohydrate. Isolated cotyledons increased in weight from 2.2613 to 2.6153 gms. and the sugar content increased from .3416 to .4684 gm. Fatty seeds on germination should show a greater quantity of oxygen taken in than of CO_2 given off. In germination there is an increase in the quantity of carbohydrate at the expense of fats. This is illustrated in the following table (Table 18).

TABLE 18

PEANUT

Age in days	*Per cent fat*	*Per cent carbohydrates other than cellulose*	*Per cent cellulose and other insoluble carbohydrates*
0	51.39	11.55	2.51
6	49.81	8.35	3.46
10	36.19	11.09	5.01
12	29.00	12.52	5.22
18	20.45	12.34	7.29
28	12.16	9.46	9.48

The principal change other than respiration going on during the germination of the peanut then is the transformation of the reserve fat into the cellulose of the cell wall.

There are many cases of the re-formation of starch from fat. This is a common occurrence in trees in spring. The transformation involves the production of glucose from glycerin and fatty acids, and the condensation of glucose to starch. In the germination of oily seeds, such as *Ricinus* seed, there is a rapid conversion of fat into glucose and starch (Fig. 45). Maquenne believed that the sugars which are produced during germination originated from unsaturated fatty acids while the saturated acids were used up by the germinating seed in respiration. It is difficult to see how this difference in use might occur if both acids were first transformed before oxidation in respiration as has usually been asumed to occur. There is a greater reactivity shown by acids of the unsaturated series, and this may account for their early disappearance by conversion into carbohydrates in germination.

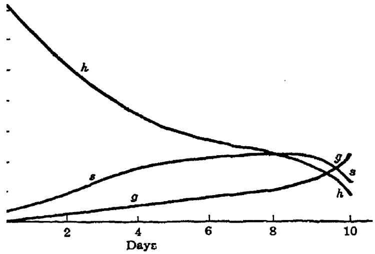
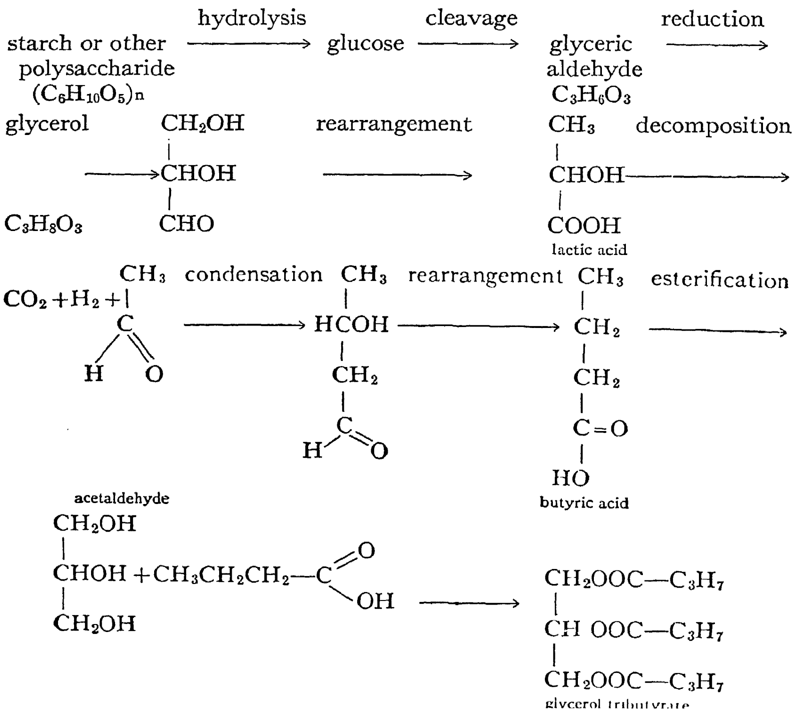


FIG. 45.—Curves representing the variations in fatty substances (h), of sucrose (s), and glucose (g) in the albumen of *Ricinus* seed during germination. (Leclerc du Sablon.)

The reactions leading to the formation of fats may be represented schematically as follows:



CHAPTER XV

THE LIPIDES

There are other fatty substances in protoplasts such as lecithin and its allies which are hardly to be considered as storage materials since they are extremely reactive and take an active part in the physiological changes in the cells. These substances may be called *cytolipoids*. They are important in cell life but are not ordinarily present in more than small quantity. They are never important as storage forms, never representing more than 1.5% of the dry weight, as in peas, and usually representing not more than 0.5%, as in the cereals. These substances are variously referred to as *lecithins*, *phosphatides*, or *phospholipins*. Probably *lipides* or *phosphatides* is to be preferred as a group name. The term *lipoid*, meaning fat-like, has been altogether too loosely applied to be specific.

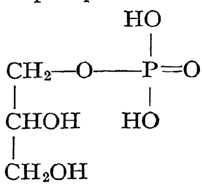
The phosphatides of plants are compounds of glycerin combined in ester linkage with two fatty acids which may be various but one of which always belongs to the unsaturated series, and one phosphoric acid group, one hydrogen of the phosphoric acid being displaced by a base which is usually choline, less frequently amino-ethyl alcohol. The groups present give the phosphatides the property of ionizing both as acids and as bases.

Lecithin is the most abundant of the phosphatides and is universally distributed in the plant kingdom. It may be represented in structural formula as follows:

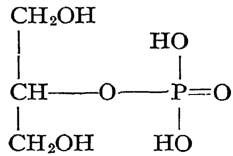
$$
\begin{array}{l}
CH_2\text{—}COO\text{—}R_1 \\
| \\
CH\text{—}COO\text{—}R_2 \\
| \\
CH_2\text{—}O \\
\quad\quad | \\
HO\text{—}P{=}O \\
\quad\quad | \\
\quad H\text{—}O\text{—}H \\
\quad\quad\quad | \\
\quad H\text{—}O\text{—}H \\
\quad\quad\quad | \\
\quad\quad\quad N{\equiv}(CH_3)_3 \\
\quad\quad\quad | \\
\quad\quad\quad OH
\end{array}
$$

lecithin

In this formula there is an asymmetrical carbon atom in the second alcohol group of the glyceryl radical. Lecithin is optically active, so the formula indicates the properties of the substance. Lecithin yields an optically active glycerophosphoric acid on cleavage, showing that the asymmetrical carbon atom is in the glyceryl group. The fatty acid radicals of lecithin may be stearic, palmitic, oleic, or linoleic acid, but there is good evidence that one of the acid radicals is always of the unsaturated series. Probably different lecithins are characterized by having different fatty acids in the molecule. It is very difficult to prepare unchanged lecithin, since the acid groups are easily oxidized. The fatty acids found seem in certain preparations to be all of the C_{18} series. Lecithin can be split on hydrolysis by alkali or lipases into fatty acids, glycerophosphoric acid, and a base. The glycerophosphoric acid may be considered a glyceryl ester of phosphoric acid which could be formed in two ways as follows:



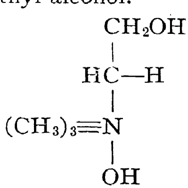
α-form asymmetrical



β-form symmetrical

Obviously the β-form does not show optical isomers since it has no asymmetrical carbon atoms, but the α-form shows dextro- and levo-rotatory forms. Probably both α-and β-forms occur in lecithin. Preparations of glycerophosphoric acid from lecithin show optical activity, so that the α-form is not absent. Since the glycerophosphoric acid is ordinarily l-rotatory there is some doubt that d-α-glycerophosphoric acid is a constituent of lecithin. It is probable that the β-form is also present in the lecithin and in some preparations it has seemed to be the predominant form.

The base contained in the molecule of the phosphatides is principally choline, as in lecithin, but there is present also the base found in cephalin, amino-ethyl alcohol.

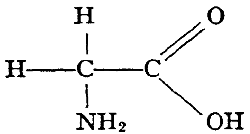


choline

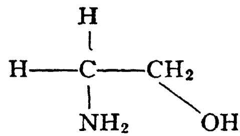
$NH_2—CH_2—CH_2—OH$

amino-ethyl alcohol

Amino-ethyl alcohol (cholamine) corresponds in structure to glycine and may be produced from this amino acid.

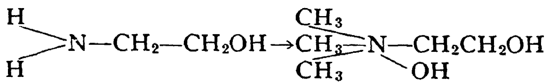


glycine



amino-ethyl alcohol

On methylation of the nitrogen, cholamine may yield choline.



cholamine choline

The phosphatide cephalin, which contains amino-ethyl alcohol, is similar to true lecithin, the only difference being in the nature of the basic group. The amino group of cephalin is free and it can be determined by the method of Van Slyke for amino nitrogen. Phosphatides containing the amino-ethyl alcohol group are found in the bean (*Phaseolus sp.*). All of the water-soluble nitrogen of lecithin is present either as choline or amino-ethyl alcohol, hence no other bases are present.

The substance lecithin, as the term is generally applied, consists of combinations of true lecithin containing choline, and cephalin containing amino-ethyl alcohol in mixture. Whether both bases are combined with the optically active l-α-glycerophosphoric acid or with the β-form, or with both, has not been determined, but the number of possible combinations is great, which might account for differences in properties of the lecithins. Differences also in the fatty acids present would account for differences in properties of these substances.

Lecithin forms slimy emulsions with water. Its colloidal solutions are easily changed in their state of aggregation by small quantities of divalent cations in particular, and to a less degree by monovalent cations. Electrolytes decrease both the osmotic pressure and the viscosity of aqueous lecithin suspensions. Lecithin may be important in determining the permeability of cells to salts on account of these properties.

Lecithin forms adsorption compounds with dyes and with albumoses. It combines with acids and bases. It forms addition compounds with certain salts of the heavy metals, such as mercury and cadmium. It forms compounds with proteins, the lecithoproteins, and it seems probable that the greater part of the lecithin of tissues exists in combination with protein. In plants, lecithin is found in combination with sugars,

much in the same manner as in the cerebrosides of animals. The sugars may be glucose, galactose, pentoses, or methylpentoses, but the galactose compound is most abundant in plants.

The phosphatides are the most labile of all the components of the colloidal protoplasm, and they play an important rôle in cell metabolism. Lecithin undergoes marked changes in properties as a result of oxidation. Owing to the ease with which it is oxidized and on account of the unsaturated fatty acid and the glycerophosphoric acid which it contains, it seems of considerable importance in respiratory processes. The action of the oxidase systems in the cell is bound up with the presence of phosphatides. The latter are therefore important in oxidative processes, the usual statement being that they act as coenzymes for the oxidase systems. Since lecithin yields glycerophosphoric acid, it may also act as a coenzyme of zymase in the alcoholic fermentation of hexoses.

The phosphatides increase rapidly during the process of ripening in seeds. On germination there is a decrease in the quantity of phosphatides as growth progresses in some plants, while in others the reverse seems to hold. The relation of quantity of phosphatide to phosphorus nutrition has not been sufficiently studied. When seeds are germinated in darkness, the phosphatides seem to decrease in quantity, while in light there is an increase.

The choline portion of lecithin is closely related to the poison, muscarine, found in the poisonous agarics (*Amanita muscaria*), and also to the betaine found in beets, and the neurine produced by the decomposition of fish and meats. The alcohol choline is not a poison, but its aldehyde muscarine is a strong poison; the acid anhydride betaine is not poisonous, but neurine is very poisonous.

$N\equiv(CH_3)_3$	$N\equiv(CH_3)_3$	$N\equiv(CH_3)_3$	$N\equiv(CH_3)_3$
$\mid$ OH	$\mid$ OH	$\mid$ OH	$\mid$ OH
CH_2CH_2OH	CH_2CHO	$CH_2C{=}O$	$C{=}CH_2$
			$\mid$
			H
Choline	Muscarine	Betaine	Neurine

It is easily seen how these substances may originate from each other by proper conditions of oxidation or reduction. Betaine and choline are both widely distributed in plants, choline being by far the more abundant. Choline is found in seeds, fruits, or other plant parts, in cocoanut endosperm, calamus root, and barley. Betaine is found in some quantity in beet roots.

Phosphatides are most abundant in tissues which have a high protein content, their abundance seeming to parallel the abundance of proteins.

CHAPTER XVI

WAXES

The fatty acids form esters with alcohols other than glycerol, and these compounds are of frequent occurrence and of physiological importance in plants. Of particular interest are the compounds of fatty acids with monatomic alcohols of high molecular weight, called *sterols*, which form the waxes. A wax in technical terms is an ester of a sterol with a fatty acid. Waxes may be either liquid or solid at ordinary temperatures. Fats differ from the waxes in that glycerol, the trihydroxyl alcohol, is always the alcohol combined in the ester, while in waxes the alcohol is always monohydroxylic. We ordinarily do not include esters of low molecular weight among the waxes but refer to them simply as *esters* or as *volatile oils.*

The common higher alcohols or sterols found in quantity in plant waxes are:

Carnaubyl alcohol	$C_{24}H_{50}O$	in carnauba wax
Ceryl alcohol	$C_{26}H_{54}O$	in Chinese wax
Myricyl alcohol	$C_{30}H_{62}O$	in beeswax

These alcohols are all of the saturated series, although unsaturated alcohols are found as constituents of waxes. These are combined into esters with various fatty acids in the common waxes as follows:

	M.-P.	
Ceryl palmitate	79° C.	in poppy wax
Ceryl cerotate	80° C.	in Chinese wax
Myricyl palmitate	72° C.	in beeswax

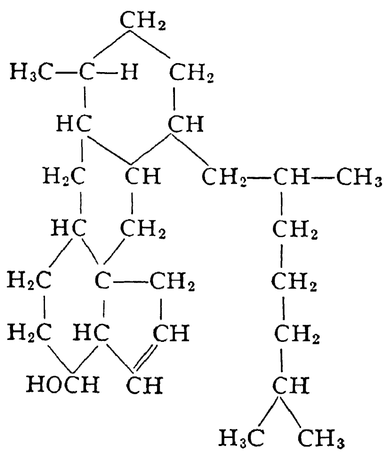
These waxes are not at all reactive; they require long boiling with alkali to effect hydrolysis. They are found in plants principally as constituents of the cuticle of leaves or fruit. One of their functions is to prevent too rapid evaporation from the plant. They are found especially abundant in plants which are adapted to xerophytic conditions, such as cacti (CACTACEÆ), euphorbias (EUPHORBIACEÆ), etc., of desert regions. The waxes also are of considerable importance in herbaceous plants which are exposed to frosts. Their function in this case is to form a waterproof coating which prevents dew from collecting on the plants, which on freezing would cause the leaves to be inoculated with the crystals and to

freeze. The presence of wax allows the tissues to undercool much below their normal freezing-point, and thus they are a protection against frost injury.

In addition to sterols there are found in the cuticle of fruits also complex hydrocarbons called *sterenes*. In the cuticle of apple these substances are of importance in determining the permeability of the epidermis to oxygen. The waxy covering of apples is of importance also in the retention of acetaldehyde and esters by the fruits, and thus is related to the incidence of storage scald. Both sterols and sterenes may be just as important as lecithin in determining the permeability of cells and membranes.

The waxes when once formed in the cuticle of leaves or fruits are not again brought into use by the plant. They are very stable substances, and are probably not used as a source of energy by organisms with the possible exception of some fungi which grow on the surface of leaves. The waxes may remain unchanged for centuries. In fact, there are remains of plants of the Devonian period, the *Calamites*, deposited in Russia, in which the coal is made up of the waxy epidermal layer of the leaves almost entirely. Peat deposits frequently are made up of a large percentage of such substances.

In addition to the above-mentioned straight-chain alcohols, the waxes of plants contain also cyclic alcohols whose compounds with fatty acids are called *phytosterols*. These substances are compounds similar to the cholesterol of animals, and were formerly taken to be isomerides of the cholesterols.



Windaus's formula for cholesterol.

There is one compound, hippocuprosterol, $C_{22}H_{54}O$, found in grasses, which may be regarded as a reduced cholesterol. The principal phytosterols are sitosterol and stigmasterol. Sitosterol, $C_{27}H_{44}O + H_2O$, occurs in the embryos of wheat, rye, and maize. Stigmasterol, $C_{30}H_{48}O$, was first found in the oil of Calabar bean, *Physostigma venenosum*. It occurs also in rape-seed oil and cocoa-butter. The presence of these compounds in many fats of plant origin has been used to detect the adulteration of butter with cheaper fats of vegetable origin, since they give different color reactions from those of the cholesterols of animal fats. Ergosterol occurs commonly in plants, and seems to be of especial interest since an activated form produced by exposure to ultra-violet light has a physiological effect identical with the antirachitic vitamine.

The physiology of the formation of waxes in plants has never been sufficiently studied. The waxes are important as coverings of fruits and vegetables. When the waxy coating is removed from many fruits, they wilt much more quickly than if the coat is left intact. The waxy covering on the surface of small fruits aids in preventing fungus infection. If strawberries are picked in the morning when the temperature is low and the wax firm, there is much less rotting than in berries which are picked in the warm part of the day when the wax is softened. Not many fungi are able to dissolve the waxy coverings of fruits or leaves. They are generally penetrated by mechanical means by appressoria, or the organism may take advantage of cracks in the wax to gain entrance into the tissue.

There occurs in the surface layers of most plants a mixture of substances which have been insufficiently studied chemically. These are commonly called *cutin* and *suberin*. The name *cutin* is given to deposits in the walls of epidermal cells particularly, while the term *suberin* is reserved for deposits in the cell walls of endodermis. Suberin is an important constituent of cork. These substances show considerable differences in physical and chemical properties. Cutin does not seem to be made up of esters, as the true waxes are, because little or no glycerin is produced on saponification of the cuticular layers. It has been fairly well proved that suberin consists of condensation products, varying in their degree of anhydration, formed from phellonic and phloionic acids and probably other acids of a similar nature. The phellonic and phloionic acids are soluble in fatty substances, but on heating they form condensation products, probably anhydrides, which are not soluble in lipoid solvents. Cutin may also be formed by such a condensation, but the constituent acids are different. Both cutin and suberin seem to be deposited as a distinct layer in the wall. In cork cells the suberin layer lies between the inner cellulose wall and the middle lamella.

PART IV

PART IV

PROTEINS

CHAPTER XVII

COMPOSITION AND FUNCTION OF PROTEINS

The name *protein* is applied to an extensive group of nitrogen-containing substances which contain the characteristic group of the amino acids, R $CHNH_2$ COOH, or anhydrides of this group. The protein molecule may contain a relatively enormous number of groups widely varying in chemical composition and properties joined to the groups of the amino acids. On account of their ability to combine with practically all classes of chemical substances found in cells, the proteins may be of primary importance in bringing about the vital reactions of metabolism. Metabolic reactions involve the combination or decomposition of parts of the various groups which are united with the proteins. The proteins form the medium for life processes. It is impossible to say that any one chemical substance is the biological unit or vital portion of a cell, for the cell itself is the smallest unit of living matter. But certainly vital processes are impossible without protein. On starvation carbohydrates and fats may be depleted, but under no condition can the protein be depleted without death.

The proteins are always present as principal constituents of protoplasm. Probably a majority of the chemical and physical properties of protoplasm are determined by its protein constituents.

The protoplasm of slime-molds represents as purely protoplasmic constituents of plants as one may obtain. In the slime-mold *Enteridium* the proteins make up at least half of the dry weight. Pure protein in the crystalline condition may exist side by side with the actively metabolizing part of the protoplasm in storage organs. Such protein reserves are not to be considered the living part of the protoplasm. It is evident that vitality is not necessarily a property of the protein alone, however complex its structure may be. Other substances are required also to produce the living cell.

Specialized portions of the protoplasm of cells, such as cytoplasm, nuclei, and plastids, may live for a time when separated from the other

constituents of the cell, yet the separate part is not complete enough in all of its reactions to continue an entirely independent existence or to show the automatic self-maintenance, growth, and reproduction characteristic of permanently living structures. The protoplasm of the cell, owing to the great complexity of its phases, takes on a new property, life, not found in the sum of the physical and chemical properties of its individual constituents. Yet each constituent of the protoplasm may determine the nature and course of the vital reactions. Certainly the proteins as they may be combined with other substances in the cell approach more closely to the chemical and physical properties of protoplasm than any other substances.

The larger proportion of crystallizable proteins have approximately the following composition:

Carbon	50–55%
Hydrogen	6.5–7.3%
Nitrogen	15–17.6%
Oxygen	19–24%
Sulphur	3–5%

Certain classes of proteins contain also phosphorus, iron, copper, and other chemical elements in smaller amounts.

The constitution of protoplasmic protein is much in doubt since in its analysis many changes may occur, so that the chemical and physical properties of the material under analysis are much different from those shown by the original protoplasmic protein. The dehydration and racemization of the proteins during the analysis or even in the plant itself may produce proteins showing widely different properties. However, it is possible to prepare certain plant proteins in a crystalline condition, and in these the composition is relatively constant. It is possible by the use of $NaCl$, $NaBr$, or NaI in the extraction process to vary greatly the relative proportions of the constituents of the protoplasm which have been classed on the basis of solubility. This probably means that the scheme of classification of the proteins which has been in use the world over and based on solubilities in part is entirely artificial. Differences in the protein solubility produced by the Cl^-, Br^-, or I^- ions would shift greatly the percentage of globulins from identical samples. A much more satisfactory classification would be upon the basis of the actual constitution of the proteins, but, unfortunately, their complexity is so great that we can only roughly approximate the relationships. The constituent amino acids yielded on hydrolysis give definite information for only a few groups. For many of the amino acids, unfortunately, the different analytical procedures may give varying results. The precipitin reactions produced by the injection of plant proteins into the blood stream of animals

indicate that there are great differences in the proteins of different species of the same genus of plants. Greater differences exist between the pro-

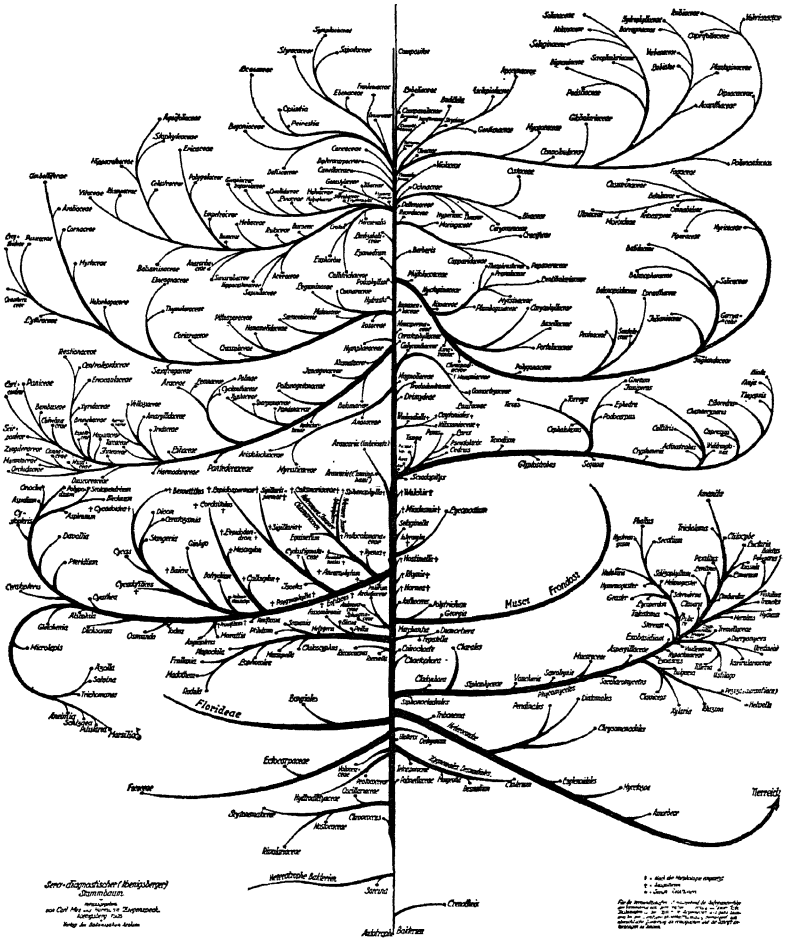


FIG. 46.—Relationships in the plant kingdom obtained by serodiagnostic methods by Carl Mez and Hermann Ziegenspeck. Königsberg, 1926. (Verlag des Botanischen Archivs.)

teins of different genera and families of plants. Mez has shown the fundamental relationships in the groups of the plant kingdom (Fig. 46).

Probably it is the expression of these differences in specific proteins of varieties of plants which we see expressed in color, in size, and in the multitudinous other characters of plants which we have been able to observe.

II. *Classification of Proteins*

With the present limitations in mind, a working classification of the proteins may be used until a better one is devised. Three main divisions may be recognized:

1. Simple proteins.
2. Conjugated proteins.
3. Derived proteins.

But in this regard may it not be asked, are not all proteins in the protoplasm conjugated, and are not all proteins undergoing analysis derived?

1. The simple proteins may be further classified into

a. Albumins.
b. Globulins.
c. Glutelins.
d. Prolamines.
e. Albuminoids.
f. Histones.
g. Protamines.

(*a*) *Albumins.* These proteins are named from their resemblance to the white of egg. It is almost a universal practice to restrict the term *albumins* to substances soluble in water and coagulated by heat. Albumins and globulins are differentiated on the basis of their solubility in half-saturated $(NH_4)_2SO_4$ solution, albumins being precipitated and globulins remaining in solution. Animal albumins are not precipitated by saturated NaCl or $MgCl_2$ solutions. Vegetable albumins are in many cases precipitated. Such vegetable albumins can be classed with albumins only on the basis of their solubility in H_2O at neutral or acid reaction and coagulation by heat. It is often difficult to decide about the solubility in water, for small quantities of salts, acids, or bases, as impurities change the solubilities greatly. As examples of albumins the following may be given:

Leucosin in cereals.
Legumelin found in various legumes.
Phaseolin in *Phaseolus vulgaris.*
Ricin, a toxalbumin of *Ricinus communis.*

Quantities of albumins are found in most other seeds.

(*b*) *Globulins.* These are proteins not soluble in distilled H_2O but soluble in neutral saline solutions. They also are commonly found in seeds. Very often it is the acid salt, not pure protein, that is soluble in salt solution and not in H_2O. When freed from acid they are soluble in water.

Animal globulins are coagulated completely by heat, plant globulins are coagulated partly, very poorly if they are free from acids. Many of these substances are easily crystallized. They have been most studied on account of the possibility of preparing them in crystalline form by dissolving them with NaCl solution and then dialyzing the extract.

As examples of plant globulins the following may be given:

Excelsin found in the Brazil-nut (*Excelsa*).
Edestin from hemp seed (*Cannabis sativa*).
Tuberin in potato (*Solanum tuberosum*).

(*c*) *Glutelins.* These proteins are not dissolved by H_2O, neutral saline solutions, or alcohols. After all other proteins are dissolved out, they can be extracted by dilute alkaline solution. Glutenin of wheat and oryzein of rice are well characterized representatives of the group. Similar proteins have been prepared also from other cereals.

d. Prolamines. These alcohol-soluble proteins were the first to be recognized in seeds. In 1805 Einhof found them in seeds of rye and barley. It is one of the groups best characterized in either plants or animals. They dissolve in 70–90% alcohol, and are precipitated by concentrated alcohol (95% or above) or dilute alcohol (50% or below). Osborne proposed to call this group the *prolamines* because all its members thus far hydrolyzed yield large quantities of proline and amide nitrogen.

Examples of the prolamines are:

Gliadin found in wheat (*Triticum vulgare*).
Hordein of barley (*Hordeum vulgare*).
Zein of maize (*Zea mays*).

In plants no representatives of albuminoids, histones, or protamines have been found.

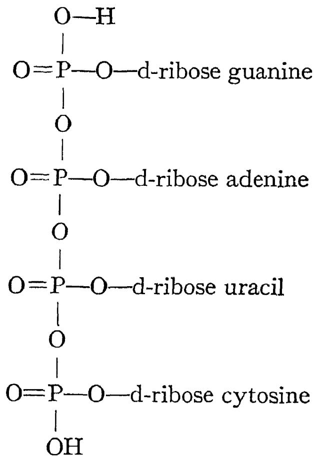
2. Conjugate proteins may be classified as follows:

a. Nucleoproteins.
b. Glucoproteins.
c. Chromoproteins.

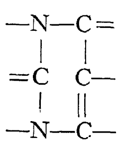
(*a*) *Nucleoproteins.* These are among the most important constituents in the cells of animals and plants. They make up the larger part of the chromatin material of nuclei. Hoppe-Seyler obtained a preparation from yeast similar to the nucleoproteins obtained from animals. The constitution of the nucleoproteins may be represented as follows:

Nucleoprotein
- Protein 1
- Nuclein
  - Protein 2
  - Nucleic acid
    - Phosphoric acid
    - Carbohydrate (pentose, ribose)
    - Purine base

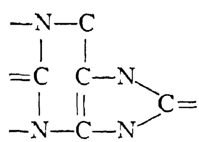
The constitution of the nucleic acid of plants is represented as follows:



It should be noted that the characteristic groups of nucleic acid are the pyrimidine nuclei of cystosine and uracil and the related purine nuclei of adenine and guanine.

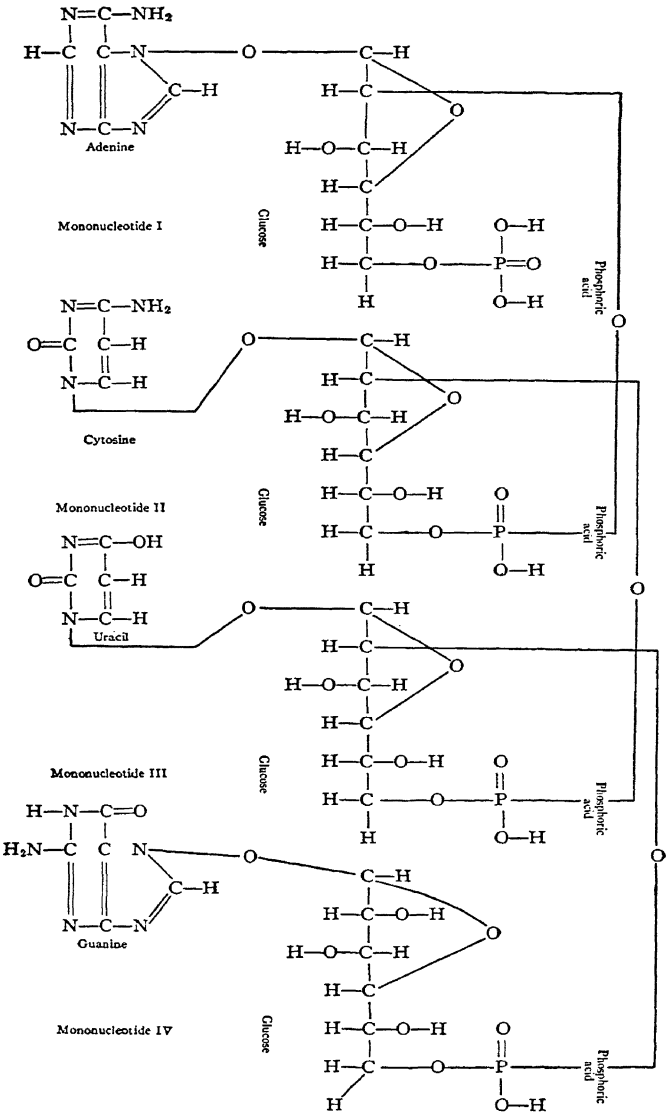


Pyrimidine nucleus



Purine nucleus

Evidently purine rings can be synthesized from other protein residues even in animals. Purine nuclei are synthesized during the development of the animal egg. They may be formed from arginine and histidine.



Plant nucleic acid

The nucleus is a center of oxidation in the cell. The nucleo proteins are characteristic nuclear constituents. The constitution of the nucleic acids, having both phosphoric acid and carbohydrate, suggests that these substances may be of importance in respiration.

(*b*) *Glucoproteins*. These are proteins containing a carbohydrate group. It has not been established definitely that they exist in plants. Plant proteins when most highly purified do not give the Molisch test for carbohydrates; consequently there is some doubt as to the existence of such compounds. Mucin of *Dioscorea sp.* may be a representative of this group.

(*c*) *Chromoproteins*. In this group there are chromatophore bodies united with the protein. Phycoerythrin and phycocyanin are examples of this class. These proteins are found in the CYANOPHYCEÆ and RHODOPHYCEÆ.

3. Derived proteins are divided into:

I. Primary protein derivatives.
   a. Proteans.
   b. Metaproteins.
   c. Coagulated proteins.

II. Secondary protein derivatives.
   a. Proteoses.
   b. Peptones.
   c. Peptids.

I. PRIMARY PROTEIN DERIVATIVES.—(*a*) *Proteans*. Edestan is an example of this group. They may be produced as anhydrides of globulins. A small amount of HCl added to edestin forms a substance insoluble in NaCl solution. It is not an acid albumin for it is not soluble in a slight excess of KOH. All seed proteins give such fractions. Proteans exist in small quantity in seeds, due to the acids present there. Edestan gives practically the same analysis as edestin. Probably it is formed by the loss of water from edestin. These protein anhydrides are important physiologically, for they represent forms in which the proteins may exist in plants and which may be produced by the action of various agents in the cell.

(*b*) *Metaproteins*. These are racemized proteins produced by the action of strong acid and alkali.

(*c*) *Coagulated proteins*. Proteins may be coagulated by various agents such as: (1) Alcohol. Plant proteins are less readily modified by alcohol than animal proteins. Zein in strong alcohol forms a gel. This probably is not true of other alcohol soluble proteins. (2) Heavy metals.

These ions produce coagulation, owing to the formation of salts of the heavy metals. (3) Heat. Proteins may be coagulated by heat. They are difficult to coagulate when in neutral solution, still more so when in an alkaline medium.

II. SECONDARY PROTEIN DERIVATIVES.—These substances are hydrolytic products of digestion by acid, or alkali, or enzymes. The groups proteoses, peptones, and peptides represent groups of decreasing complexity. Peptic digestion gives chiefly proteoses and peptones. Tryptic and ereptic digestion gives amino acids by the cleavage of these primary digestion products. Proteoses are precipitated by saturated $(NH_4)_2SO_4$ solution, peptones are not.

The distribution of the fractions of the protein of whole wheat is as follows:

	Per cent dry wt.	
Glutamin	4.68	
Gliadin (alc. sol.)	3.96	
Globulin	.62	Probably mainly from the embryo
Albumin	.39	
Proteose	.21	

	Embryos only of wheat *Per cent dry wt.*
Glutamin	0
Albumins	10
Globulins	5
Proteose	3

III. *Classification of Amino Acids*

The amino acids are the building stones of the proteins. In the whole organic world twenty-two amino acids have been recognized. Some of these are more common in animals than in higher plants, although thorough search through the whole plant kingdom might show the presence of all of the amino acids. The amino acids are classified as follows:

1. *Aliphatic compounds*
   a. Monocarboxylic mono-amino acids
      Glycine, or α-amino-acetic acid
      CH_2NH_2COOH
      d-Alanine, or d-α-aminopropionic acid
      $CH_3—CHNH_2—COOH$
      d-α-Aminobutyric acid

$$\begin{array}{ccccccc} & & H & & H & & H \\ & & | & & | & & | \\ H & — & C & — & C & — & C—COOH \\ & & | & & | & & | \\ & & H & & H & & NH_2 \end{array}$$

(questionable)

d-Valine, or d-β-dimethyl-α-aminopropionic acid

$$\begin{array}{l} CH_3 \\ \quad \rangle CH{-}CHNH_2\ COOH \\ CH_3 \end{array}$$

l-Leucine, or l-β-dimethyl-α-aminobutyric acid

$$\begin{array}{l} CH_3 \\ \quad \rangle CH{-}CH_2{-}CHNH_2{-}COOH \\ CH_3 \end{array}$$

d-Isoleucine, or d-β-methylethyl-α-aminopropionic acid

$$\begin{array}{l} C_2H_5\quad H\quad H \\ \qquad\quad\ |\quad\ | \\ \qquad \rangle C{-}CNH_2{-}COOH \\ CH_3 \end{array}$$

d-Norleucine, or d-α-aminocaproic acid

$CH_3{-}CH_2{-}CH_2{-}CH_2{-}CHNH_2{-}COOH$

l-Serine, or l-β-hydroxy-α-aminopropionic acid

$CH_2OH{-}CHNH_2{-}COOH$

Cysteine, or β-thio-α-aminopropionic acid

$CH_2HS\ CHNH_2\ COOH$

b. Dicarboxylic mono-amino acids

l-Aspartic acid, or l-α-aminosuccinic acid

$COOH{-}CH_2\ CHNH{-}COOH$

Its amide is asparagin

$CONH_2{-}CH_2{-}CHNH_2{-}COOH$

d-Glutamic acid, or d-α-aminoglutaric acid

$COOH{-}CH_2{-}CH_2{-}CHNH_2{-}COOH$

Its amide is glutamine

$CONH_2{-}CH_2{-}CH_2{-}CHNH_2{-}COOH$

β-Hydroxyglutamic acid

$COOH{-}CH_2{-}CHOH{-}CHNH_2{-}COOH$

c. Monocarboxylic di-amino acids

d-Arginine, or d-δ-guanidine-α-aminovaleric acid

$$\begin{array}{l} \qquad\qquad \diagup NH_2 \\ HN{=}C \\ \qquad\qquad \diagdown NHCH_2{-}(CH_2)_3{-}CHNH_2{-}COOH \end{array}$$

Lysine, or α-ε-di-aminocaproic acid

$NH_2CH_2{-}CH_2{-}CH_2{-}CH_2{-}CHNH_2{-}COOH$

Hydroxylysine, or α-ε-amino-β-hydroxycaproic acid

$CH_2{-}NH_2{-}CH_2{-}CH_2{-}CHNH_2{-}COOH$

d. Dicarboxylic di-amino acid

Cystine, or di-(β-thio-α-aminopropionic acid)

$$\begin{array}{lcl} CH_2{-}S & {-} & S{-}CH_2 \\ | & & \quad\ | \\ CHNH_2 & & CHNH_2 \\ | & & \quad\ | \\ COOH & & COOH \end{array}$$

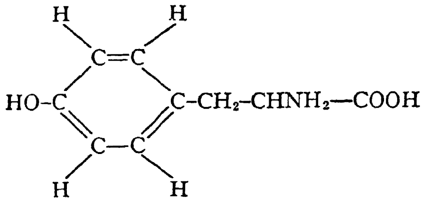
2. *Aromatic compounds*

Mono-amino monocarboxylic acids

l-Phenylalanine, or l-β-phenyl-α-aminopropionic acid

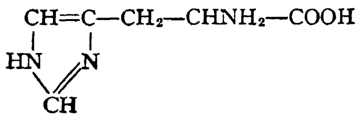
$C_6H_5CH_2—CHNH_2—COOH$

l-Tyrosine, or l-β-parahydroxypenyl-α-aminopropionic acid



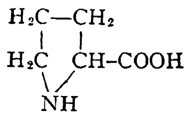
3. *Heterocyclic compounds*

l-Histidine, or l-β-imidazol-α-aminopropionic acid

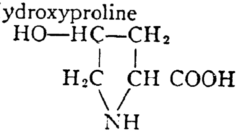


Imino acids

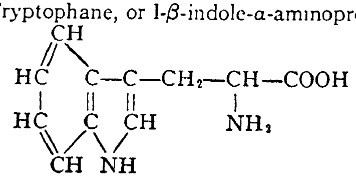
Proline, or α-pyrollidine carboxylic acid



Hydroxyproline



l-Tryptophane, or l-β-indole-α-aminopropionic acid



CHAPTER XVIII

SYNTHESIS OF PROTEINS

I. *Sources of Amino Acids*

The large number of derivatives of propionic acid should be noted. Evidently some three-carbon unit is of importance in the formation of these acids. The ease of replacement of OH by NH_2 suggests the probability of lactic acid derived from carbohydrate cleavage as a precursor of these three-carbon amino acids. The animal body possesses the property of forming amino acids by substitution from the corresponding hydroxy acid. The ketonic acids may be utilized similarly.

II. *Ionization of Amino Acids*

The carboxyl groups of the amino acids may form salts with basic ions, or with amides, or with amino groups. The basic groups (amino—NH_2, imide=NH, amide $CONH_2$) may combine with acids. In addition there are —OH groups in certain amino acids which may form salts or esters. The phenolic —OH groups may form esters and salts. The guanidine group is basic and forms salts. One hydrogen of tryptophane is very reactive. The —SH group of cystine is very reactive and forms esters, thioesters, and ethers.

III. *Amino Acid Content of Proteins*

The great variety of proportions of the different amino acids in proteins from different plants can be judged from the following table:

	I *Albumins*	*II* *Globulins*			*III* *Gliadins*		*IV* *Glutelins*
Proteins	*Leucosin* (*wheat*)	(*a*) *Edestin* (*hemp*)	(*b*) *Squash seeds*	(*c*) *Glycinin* (*soybean*)	(*a*) *Wheat gliadin*	(*b*) *Zein* (*maize*)	*Glutelin* (*wheat*)
THE VEGETABLE PROTEINS							
Glycine.........	0.9	3.8	0.6	1.0	0.0	0.0	0.9
Alanine.........	4.5	3.6	1.7	....	2.0	9.8	4.7
Valine..........	0.2	+	0.3	0.7	3.4	1.9	0.2
Leucine.........	11.3	20.9	7.3	8.5	6.6	19.6	6.0

	I *Albumins*	II *Globulins*			III *Gliadins*		IV *Glutelins*
Proteins	*Leucosin (wheat)*	(*a*) *Edestin (hemp)*	(*b*) *Squash seeds*	(*c*) *Glycinin (soybean)*	(*a*) *Wheat gliadin*	(*b*) *Zein (maize)*	*Glutelin (wheat)*
THE VEGETABLE PROTEINS							
Isoleucine	....	....	....	....	....	....	.....
Phenylalanine	3.8	2.4	3.3	3.9	2.4	6.6	2.0
Tyrosine	3.3	2.1		1.9	1.2	3.6	4.3
Serine	?	0.3			0.2	1.0	0.7
Cystine	....	0.3	0.2		0.5	....	0.02
Proline	3.2	1.7	2.9	3.8	13.2	9.0	4.2
Oxyproline	....	2.0					
Aspartic acid	3.4	4.5	3.3	3.9	0.6	1.7	0.9
Glutaminic acid	6.7	6.3	12.4	19.5	43.7	26.2	23.4
Tryptophane	+	+	+	....	1.0	0.0	+
Arginine	5.9	11.7	14.4	5.1	3.2	1.6	4.7
Lysine	2.8	1.0	2.0	2.7	0.2	0.0	1.9
Histidine	2.8		2.6	1.4	0.6	0.8	1.8
Ammonia	1.4		1.6	2.6	5.2	3.6	4.0
	50.2	61.7	55.9		[illegible].0	85.4	59.72
Colorimetric determinations							
Tryptophane	4.76	2.48	3.01	1.66	0.70–1.09	0.0	1.72
Cystine	3.29	0.97	1.38	1.12	1.42–1.76	0.85	1.46
Total sulphur		0.880	.....	0.710	1.027	0.600	....
Cystine sulphur		0.347	.....	0.320	0.619	0.212	....

The individuality of the protein is determined not only by the number and kind of amino acids which it contains but also by the manner in which these are joined together. From their reactions it is assumed that 10–25% of the nitrogen is present in proteins in the amide linkage, R—$CONH_2$, and about 60% in peptide linkage.

IV. *Sources of Nitrogen for Amino Acid Formation*

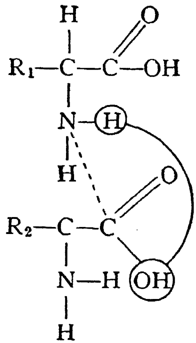
Nitrates and ammonium salts in the soil are the principal source of the nitrogen of plants. Of the two groups, the nitrates are by far the more important, since in most habitats they are the most abundant form of the soil nitrogen which is available to plants. The nitrates may be traced from the roots to all parts of the plant. They are especially abun-

dant in rapidly growing regions of the plant where protein synthesis is taking place. They are found in leaves, and in some texts of plant physiology the statement is made that the nitrates are built up into amino acids and proteins in the leaf. This statement is supported frequently by data showing that the disappearance of nitrates occurs in the green parts of variegated leaves but not in the white parts which contain no chlorophyl. These data are true, but the differences in reduction of nitrates seems to be dependent rather upon carbohydrates which are formed in the chlorophyl-bearing areas and not formed in the colorless parts. The reduction of nitrates to amino acids may occur in any part of the plant in which carbohydrates are undergoing change. Seedlings never exposed to light at all still assimilate nitrates and form proteins. The formation of protein from nitrates then is dependent upon carbohydrate and is not limited to cells which assimilate CO_2 by photosynthesis. When there is insufficient carbohydrate formation, as in chlorotic leaves, the nitrates may accumulate. Where carbohydrates are in excess, the nitrates may be exhausted. Either condition leads to abnormal metabolism. Kraus and Kraybill have shown that a number of physiological processes are dependent upon a proper balance between the carbohydrates and nitrogen constituents or upon the C/N ratio.

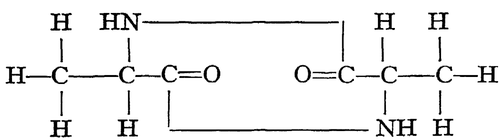
V. *Linkages between Amino Acids*

The proteins are synthesized from amino acids which may be joined together in peptide linkage. $-\underset{\underset{\displaystyle O}{\|}}{C}-\underset{\underset{\displaystyle H}{|}}{N}-$ The peptide linkage between the amino acids is from the carbon of the carboxyl group to the nitrogen of the amino group. Each amino acid contains at least one carboxyl group and one amino or imino group. When the peptide linkage has been formed there is still left in the molecule one free amino or imino group and a free carboxyl group, either of which may react to form further condensation products with other groups with the splitting off of water. Of all the possible places in the amino acid molecule in which the amino group might be situated, the attachment to the α-carbon adjacent to the carboxyl group is preferred. In fact, mono-amino acids with the amino group in other positions do not occur in plants. When once a single amino group has been introduced into the α position, another group may be found on the ϵ-carbon as in lysine, α-ϵ-di-aminocaproic acid. When amino acids are joined to such a di-amino acid, the resulting peptide may have a branched complex carbon chain. In the simpler mono-amino acids the linkage is to either the α-amino group or to a carboxyl group. There may be branched chains when the linkage involves

dicarboxylic acids, or their amides, and amino acids containing the imino group (=NH).

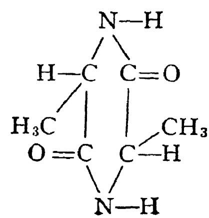


The peptide linkage may be enolized by alkali into a form resistant to tryptic hydrolysis $=\underset{\mathrm{OH}}{\underset{|}{\mathrm{C}}}-\underset{\mathrm{H}}{\underset{|}{\mathrm{N}}}-$



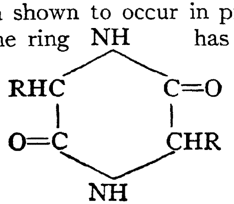
alanylalanine

or

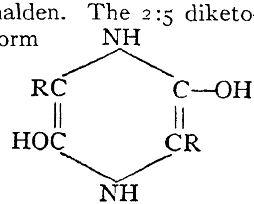


dimethyldiketopiperazine

The formation of internal anhydrides other than of the peptide type has been shown to occur in proteins by Abderhalden. The 2:5 diketopiperazine ring



has a possible enolic form



The many physical changes which proteins undergo are probably associated with the formation or opening of such anhydride linkages.

The proteins are evidently very unlike each other both with regard to

the nature of the linkage and the kind of the amino acids which they contain. This multitude of differences which may occur possibly accounts for the exceedingly variable types of plants which there are, and for the differences in the physiology of the different species which lead to the production of so great a number of plant products.

The really remarkable thing about the relationships of plant proteins is not that proteins of various great groups are related, but that throughout countless ages the syntheses of proteins has always followed the same path, producing proteins of the same constitution and precipitin reactions out of the enormous number of other combinations which it is possible to synthesize. These combinations of proteins peculiar to certain species of plants, lead to the production of the same types of storage products and to the type of metabolism to which each species is peculiarly adapted. The number of possible combinations of 22 amino acids in proteins is enormous, yet the number of species of plants is relatively limited.

CHAPTER XIX

SYNTHESIS OF PROTEINS—*Continued*

I. *Synthesis of Protein Constituents*

The proteins are synthesized from exactly the same amino acids which they yield on hydrolysis. Emil Fischer (Fig. 47) devised a method

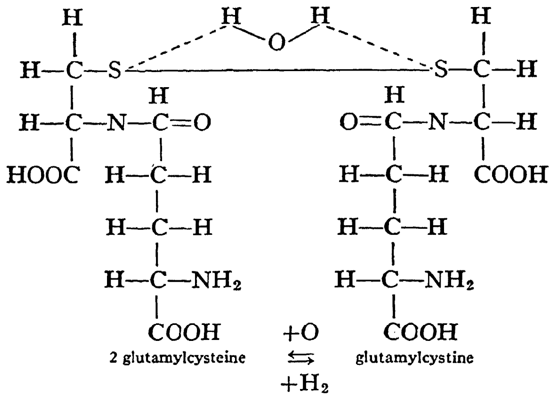
FIG. 47.—Emil Fischer, 1852–1919.

"The structure of polypeptides as a rule can be drawn directly from synthesis; this holds especially for the derivatives of the mono-amino acids, to which principally the investigations have so far extended. The stereochemistry of the class is somewhat more complex. With the exception of glycocoll, all amino acids that up to the present have been observed in protein materials, and with which we are principally concerned in these syntheses, contain an asymmetrical carbon atom. The number of these in the polypeptide corresponds accordingly to the number of amino acids bound in anhydride-like linkage (with the exception of glycocoll), and the total number of the individual optical isomers is given by the well-known Van't Hoff formula, 2^n."

Untersuchungen über Aminosäuren, Polypeptide und Proteïne.

of fractional distillation which has been of great value in the identification of protein constituents. The proteins of animals fed upon proteins which are deficient in certain amino acids will be found to lack those amino acids. The animal body is in many cases unable to synthesize all of the amino acids which it requires. It must obtain these amino acids already formed from plants either directly or indirectly. Particularly is this true of lysine, cystine, histidine, and tryptophane. Evidently certain amino acids have particular functions in metabolism and may not be replaced by other amino acids. Arginine is especially concerned in

cell proliferation. The heads of sperms are especially rich in basic amino acids, and it is their function to induce cell division. Cysteine is necessary for the formation of glutathione, or glutamylcysteine, a substance of great importance in cell oxidations.



On being oxidized the dipeptide glutamylcysteine, or glutathione, is condensed with another molecule to form diglutamyl cysteine or glutamylcystine.

Green plants have the ability to synthesize from inorganic forms of nitrogen all of the amino acids which they require. The synthesis of protein in plants then is concerned principally with the synthesis of the individual amino acids. The condensation of these into protein may be brought about by all organisms. Animals when fed free amino acids may synthesize their protein from them, and they seem able likewise to manufacture certain amino acids such as glycocoll when they are not present in the diet. This indicates that some amino acids are easily formed while others may be formed only under the peculiar conditions found in plants and which are not found in animals.

According to Erlenmeyer glycine, the simplest amino acid, may originate from glyoxylic acid. Glyoxylic acid is commonly found in plants, especially in fruits. It may originate from the reduction of oxalic acid.

$$\underset{\text{oxalic acid}}{\begin{array}{c} COOH \\ | \\ COOH \end{array}} \rightarrow \underset{\text{glyoxylic acid}}{\begin{array}{c} CHO \\ | \\ COOH \end{array}}$$

Two molecules of glyoxylic acid may react with one molecule of ammonia, producing formylglycine, water, and carbon dioxide. The formylglycine then reacting with water may form glycine and formic acid.

$$\begin{array}{cccc} 2\text{CHO} & & \text{CHNH}_2\text{CHO} & \\ | & +\text{NH}_3 \rightarrow & | & +\text{H}_2\text{O} + \text{CO}_2 \\ \text{COOH} & & \text{COOH} & \\ \text{glyoxylic acid} & & \text{formylglycine} & \end{array}$$

$$\begin{array}{cccc} \text{CHNH}_2\text{CHO} & & \text{CH}_2\text{NH}_2 & \\ & +\text{H}_2\text{O} \rightarrow & | & +\text{HCOOH} \\ \text{COOH} & & \text{COOH} & \\ \text{formylglycine} & & \text{glycine} & \text{formic acid} \end{array}$$

There is no reason why the combination of glyoxylic acid with ammonia may not go directly to glycine and formic acid.

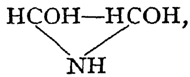
Since nitrates are the principal nitrogen source for protein formation by plants, the reactions which nitrates may undergo may give some idea as to how their transformation to amino acids may occur. In the transformation of nitrate to amino acid the nitrogen is changed from the fully oxidized to the fully reduced condition. On exposure to violet light, potassium nitrate may be split to oxygen and potassium nitrite. Frequently nitrates are reduced by simultaneous oxidation and reduction with the oxidation of another compound by the Cannizzaro reaction. In such reactions Baudisch has shown that iron compounds and magnesium compounds are of importance since they act as catalysts. Potassium nitrate may be reduced to potassium nitrite by such reactions. Baudisch thought that the nitrite in the presence of methyl alcohol in daylight was reduced to the hyponitrite. The methyl alcohol was simultaneously oxidized to formaldehyde. The hyponitrite is exceedingly reactive and at once forms with the formaldehyde, the potassium salt of formhydrox-

$$\text{KNO}_3 \rightarrow \text{KNO}_2 + \text{O}$$

$$\text{KNO}_2 + \text{CH}_3\text{OH} \rightarrow \text{KNO} + \text{HCHO} + \text{H}_2\text{O}$$

$$\begin{array}{lcc} \text{KNO} & +\text{HCHO} \rightarrow & \text{HCOH} \\ \text{potassium hyponitrite} & & \| \\ & & \text{NOK} \\ & & \text{potassium salt of formhydroxamic acid} \end{array}$$

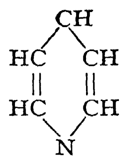
This is a photochemical reaction brought about only in the presence of light. Baly has shown that a variety of substances can be produced from formhydroxamic acid. It may lose oxygen and form a compound which is to be considered as a hydrate of hydrocyanic acid, $\begin{array}{l}\text{HC—OH}\\ \|\\ \text{N—H}\end{array}$, which will condense with more fomaldehyde to produce a labile ring compound



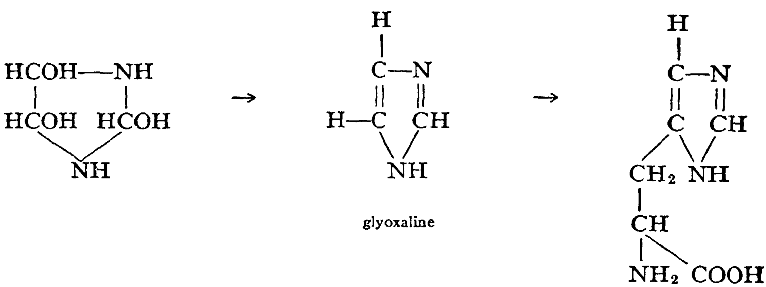
which by intramolecular rearrangement may give glycine, CH_2NH_2COOH. Alpha-amino acids have been produced both by Baudisch and by Baly from formhydroxamic acid and formaldehyde under the action of ultra-violet light. Formhydroxamic acid, with the production of the intermediate compound

may condense with three or four molecules of formaldehyde in the presence of ultra-violet light of a proper wave-length to produce pyrrol and pyridine derivatives.

pyrrol



pyridine



glyoxaline

histidine

Glyoxaline is formed from two molecules of formhydroxamic acid and one of formaldehyde. Both this compound and its substituted amino acid, histidine, have been found by Baly in the products of the action of ultra-violet light on mixtures of carbon dioxide, water, and nitrates.

Plants may produce amino acids in parts not exposed to light; in fact, they may be produced in plants which have never been exposed to light at all. Therefore the line of protein synthesis given by Baly is probably not the only one followed in plants. Baly's scheme requires the synthesis of nitrogen to occur in leaves where there is light exposure. This is a weak point in his argument, for amino acids are regularly synthesized in plants without any light exposure.

The cyanides have long been considered to be of importance as intermediates in protein synthesis. They may be produced by the reduction of nitrates. The hydrate of hydrocyanic acid is one of the intermediate compounds in Baly's scheme of amino acid synthesis. This might easily be formed by the combination of one molecule of water with a molecule of hydrocyanic acid. Hydrocyanic acid is commonly found in plants in the form of glucosides. The glucosides then may easily yield HCN and monosaccharides which could be split to carbon chains of various lengths required for the synthesis of the nitrogen bases, equally as well as these could be formed from condensation of several molecules of formaldehyde. The evidence given by Spoehr that formaldehyde is not produced in plants by photosynthesis would indicate that Baly's scheme of reactions, although capable of producing amino acids, may not be followed in plants. Baudisch held that the synthesis of amino acids, although carried out in darkness, might still be a photochemical process in the main reaction and that the reactions in darkness might be abnormal. This could certainly not be true of fungi which function normally in darkness. Evidently the process is not necessarily photochemical. The greater synthesis of protein in leaves under illumination may be dependent merely upon the carbohydrates produced thereby.

II. *Ionization of Proteins*

Proteins may ionize either as cations or as anions, owing to the presence of basic groups either amino ($—NH_2$), imino ($=NH$), or amide ($—CONH_2$), and because they possess free carboxyl groups, $—COOH$. The electrical charge on the ionized protein is determined by the hydrogen-ion concentration of the solution in which it is dispersed. The point on the pH scale at which equal numbers of molecules of protein bear positive and negative charges is known as the *isoelectric point*. For basic proteins the isoelectric point is on the alkaline side of neutrality, for acid proteins on the acid side. The cell sap reactions of most plants is slightly

acid, only a few showing alkaline reaction. The acid range of most cells covers the range of the isoelectric points of the albumins and globulins.

The basic proteins, the histones, and protamines are not strongly represented in plants. These proteins are high in di-amino acid content. The approximately neutral proteins, albumins and globulins, are prominent in plant protoplasm. These have fairly high content of di-amino acids, balanced by equally high content of dicarboxylic amino acids. The phosphoproteins are especially acidic in character because they have a high content of dicarboxylic amino acids. The isoelectric point of the protein then is an important indication of its constitution.

The isoelectric point of proteins is of importance in cell activities because it is found that at this hydrogen-ion concentration the protein shows a minimum swelling, minimum solubility, maximum viscosity of its solutions, and maximum instability toward electrolytes. At the isoelectric point proteins are most easily precipitated by alcohol. The isoelectric point varies greatly for various proteins. It seems that the protoplasmic acidity is not usually far from the average isoelectric point of the proteins. Possibly the ionization of the protein as cation or anion is associated with the uptake of other ions from solutions by the cell. In tissues there seem to be demonstrable differences between the acidities of certain cells and even of different parts of the same cell. It would seem that the basic or acidic ions of salts, dyes, etc., should be taken up by different proteins of the cell, depending upon the acidity of the region concerned. This is commonly demonstrable in tissue staining. Changes of acidity in parts of the cell should lead to redistribution of the salt and other constituents of the cell. The intake or excretion of water is probably also regulated by the responses of the proteins to changes in acid or salt content.

III. *Size of Protein Molecules*

The size of the protein molecule is rather difficult to determine. Estimates by the method of freezing-point depression and by osmotic pressure measurements indicate a very large molecule. Adair found for hemoglobin a molecular weight of 67,000. Sörensen found that the colloidal particles of egg albumin were single protein molecules having a minimum molecular weight of 33,800. An indirect method of determining the size of the molecule is by determinations of sulphur, iron, or other groups. Minimal combining weights of the proteins are given in the following table:

Protein	*Minimal combining weight*
Zein	19,400
Gliadin	20,700
Edestin	29,000
Glutenin	36,300

CHAPTER XX

CLEAVAGE OF PROTEINS

I. *Protein Analysis*

A good method to learn the constitution of the proteins is to determine the relative proportions of the constituent amino acids. Protein hydrolysis by acids yields the constituent amino acids in condition for a fairly high percentage recovery by analysis. Applications of the methods of Van Slyke, Hausmann, Foreman, Dakin, Schryver, and E. Fischer have yielded very important results on the composition of proteins.

The early methods of E. Fischer involved the separation of the mono-amino acids by esterification and fractional distillation. Although such fractional separations are by no means as quantitative as desired, better methods for the estimation of the mono-amino acids have not been devised.

The method of Van Slyke for the estimation of the mono-amino and di-amino acids by their reactions with nitrites in acid solution is of great value and is quantitative. The formol titration methods of Hausmann have been much used in the estimation of the different protein fractions.

Dakin's method leads to the separation of the protein units into five groups. These are:

1. The mono-amino acids, extracted by butyl alcohol, but insoluble in ethyl alcohol.
2. Proline, extracted by butyl alcohol, and soluble in ethyl alcohol.
3. Diketopiperazines, extracted by butyl alcohol, but sparingly soluble in ethyl alcohol or in water.
4. Dicarboxylic acids, not extracted by butyl alcohol.
5. Di-amino acids, not extracted by butyl alcohol, but separated from (4) by precipitation with phosphotungstic acid.

The method is summarized below:

Protein

↓ Boil for 10 to 16 hours with 10 times its weight of 25 per cent sulphuric acid.

Amino acid mixture

↓ Dilute and neutralize with baryta. Filter, concentrate moderately. Filter again to remove tyrosine.

Filtrate	Separation of tyrosine.

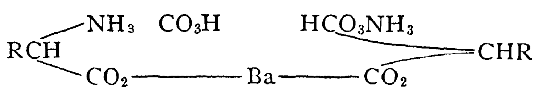
↓ Neutralize to litmus, concentrate till leucine begins to separate. Remove to extractor. Extract 36 hours. Filter.

Groups (1), (2) and (3) in extraction vessel. Group (3) separates slowly on standing.	(4) and (5) in residue. Separate with phosphotungstic acid.

↓ Filter off diketopiperazines. Evaporate to dryness. Take up with ethyl alcohol.

Proline	Residue of mono-amino acids. Separate by esterification.

Kingston and Schryver make use of the property of amino acids when dissolved in aqueous alcohol of forming with barium hydroxide and carbon dioxide crystalline carbamates of the type



The method is said to be adaptable to small quantities of material and to give accurate estimations of glycine, dicarboxylic, and di-amino acids, proline, and hydroxyproline.

A summary of the method is given below.

Gelatin
↓ Hydrolysis with H_2SO_4
Amino acid solution
+ baryta

I. Precipitate
Barium aspartate

Filtrate
Solution of barium salts
+ alcohol

II. Precipitate
Barium dicarboxylates

Filtrate
Solution of barium salts
+ CO_2 + baryta

Precipitate
Barium carbamates
extraction with ice-cold water

Filtrate
Peptide (?)
hydrolysis
IV. Proline

III. Precipitate
Barium carbamates
boiling water
↓
Glycine

Filtrate
Solution of carbamates
+ heating
↓
Amino acid solution
phosphotungstic acid

V. Precipitate
Di-amino acids

VI. Filtrate
Mono-amino acid solution
evaporation

(a) Crystalline precipitate. Leucin

Syrup
+ absolute alcohol

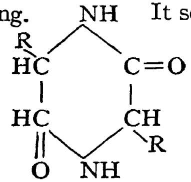
(b) Filtrate
↓
Proline

(c) Precipitate containing { alanine, phenylalinine, hydroxyproline, leucine (remains of), serine

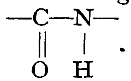
II. *Proteolytic Enzymes*

The hydrolysis of the crystalline proteins of seeds by the enzymes naturally occurring in plant tissues has yielded important information on the larger groups in the protein molecule. There are three classes of proteolytic enzymes found in plants. These are the peptases, tryptases,

and ereptases. Peptase acts upon natural protein and cleaves it to proteoses and peptones. Peptase has no action on peptones or peptides. According to Abderhalden, the natural proteins possess a cyclic structure like that of the piperazine ring.



It seems that the action of the peptases is to cause cleavage in this ring. Peptases do not act upon the peptide linkage



. The proteoses are early products of protein

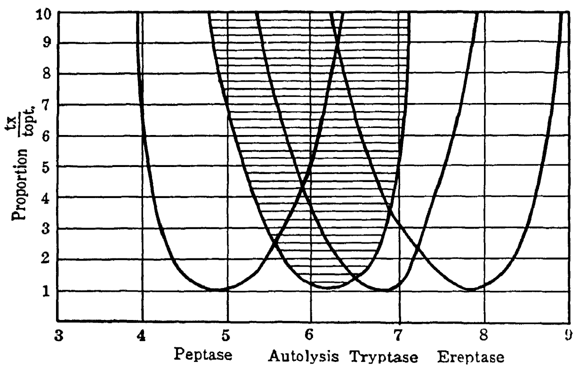


FIG. 48.—Activity of yeast proteases at various pH. (After Dernby.)

cleavage and represent large aggregates. They give the biuret test. The peptones are products of further cleavage which still give the biuret test.

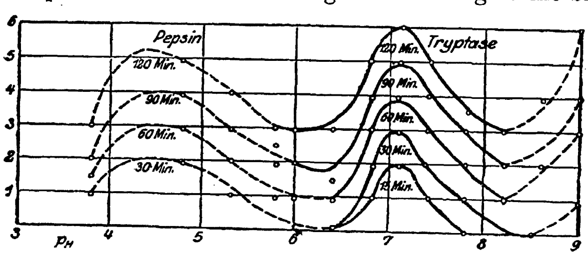


FIG. 49.—Relation of the acidity to the activity of yeast proteases. (After Dernby.)

Proteoses and peptones are found in plants, particularly during germination in seeds which are rich in protein. They are rather large aggregates of sufficiently high molecular weight to make them almost indiffusible. They are not important as translocation forms of protein since they

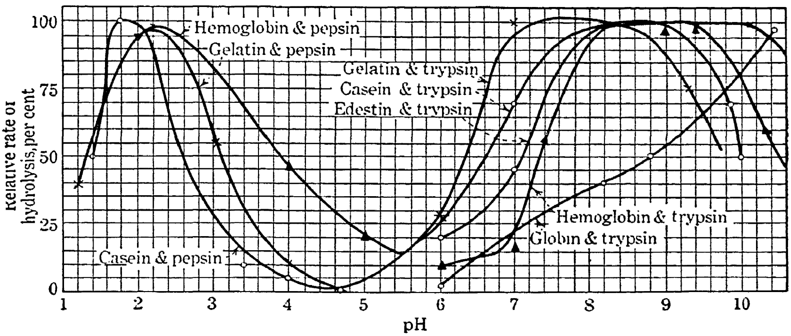


FIG. 50.—Relation of the acidity to the activity of proteases on various substrates. (Northrop.)

diffuse scarcely further than a few cells without cleavage to their constituent amino acids. The ereptases act upon peptones to produce amino acids. The tryptases give as end-products polypeptides and amino acids.

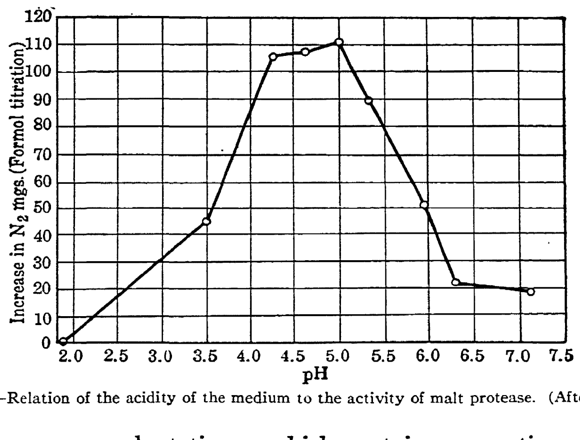


FIG. 51.—Relation of the acidity of the medium to the activity of malt protease. (After Adler.)

There are many plant tissues which contain very active proteolytic enzymes. The pawpaw fruit (*Carica papaya*) contains active proteolytic enzymes. The papain of commerce is prepared from this fruit. The pineapple fruit has very active proteolytic enzymes.

The acidity required for the action of these proteases is considerably different, as shown by the graphs (Figs. 48, 49). Pepsin of animal origin requires a strongly acid medium and shows a maximum activity at about pH 2 (Fig. 50). Peptic action in plants may proceed at lower acidities (Figs. 51, 52). Trypsin shows a maximum activity at about pH 7.

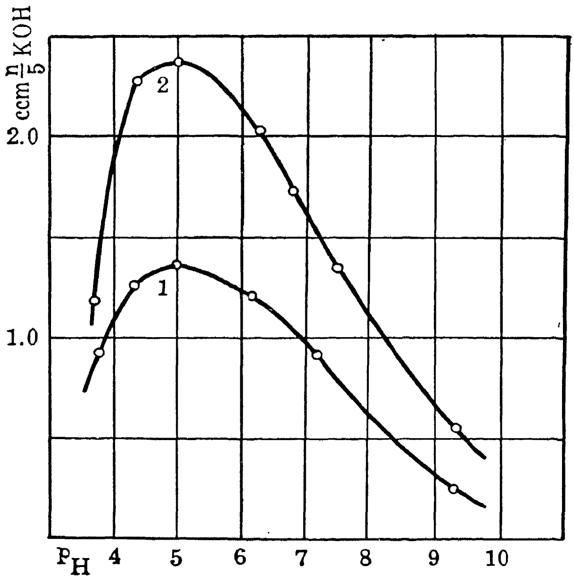


FIG. 52.—Relation of the acidity to the activity of papain (1) and papain-HCN (2). (After Willstätter.)

Ereptase acts in a still more alkaline medium, showing a maximum activity at pH 8.

If the hydrolysis and synthesis of proteins can be represented generally by the scheme

$$\text{anhydride} \underset{(3)}{\leftrightarrows} \text{polypeptide} \overset{(1)}{} \text{amino acids}$$

the following possibilities suggest themselves:

That acids catalyze reactions (1) and (3); reaction (2) only to a very slight extent.

That erepsin (and probably trypsin) catalyzes reaction (1); reaction (2) rather more than acid; reaction (3) not at all.

That pepsin has no catalytic effect on (1), but a marked effect on (2) and (3).

The conditions which determine the direction of the reaction whether to synthesize or to hydrolyze proteins is dependent upon the acidity of the medium and upon the presence of water. Desiccation of cells favors protein synthesis. The absorption of water by dry seeds favors protein hydrolysis.

The formation of proteins from their constituent amino acids seems to be brought about by the same enzymes which cause proteolysis. The action of pepsin is to accelerate the establishment of equilibrium in the reaction: protein$\rightleftarrows$polypeptide. At pH 1.7 the equilibrium point is reached when the protein is nearly all hydrolyzed. However, if the acidity is readjusted to pH 4.0 there will be resynthesis of protein demonstrable in three days. This protein can be precipitated by trichloracetic acid, whereas the digestion mixture of peptides was not precipitable before the acidity was decreased. It seems possible then to get either synthesis or hydrolysis merely by shifting the acidity of the medium when pepsin is present with a protein. In like manner the synthetic action of trypsin can be demonstrated. At pH 5.7 trypsin catalyzes the formation of anhydride from polypeptides produced by peptic digestion in the reaction: anhydride→polypeptide→amino acid. At pH 8, however, the principal action is to cause the cleavage of polypeptide to amino acids. At pH 5.7 also there is some cleavage of polypeptide to amino acid coincident with the synthesis of higher anhydride.

III. *Protein Decomposition*

In the decomposition of protein by cells, there may be a removal of the carboxyl groups or of the amino groups or of both. Decarboxylation produced by certain bacteria may lead to the production of organic amines which are usually substances with strong offensive smell and frequently with marked physiological effect. If ingested with food, these amines may be the cause of severe poisoning. In bacterial deamination the organic acids produced may cause sourness, or offensive odors if butyric acid is produced. When both decarboxylation and deamination occur, free hydrocarbons are produced, which generally are harmless but have unpleasant odors. Such reactions are produced characteristically under anaërobic conditions.

GENERAL PROTEIN METABOLISM

I. *Transformation of Protein to Carbohydrate*

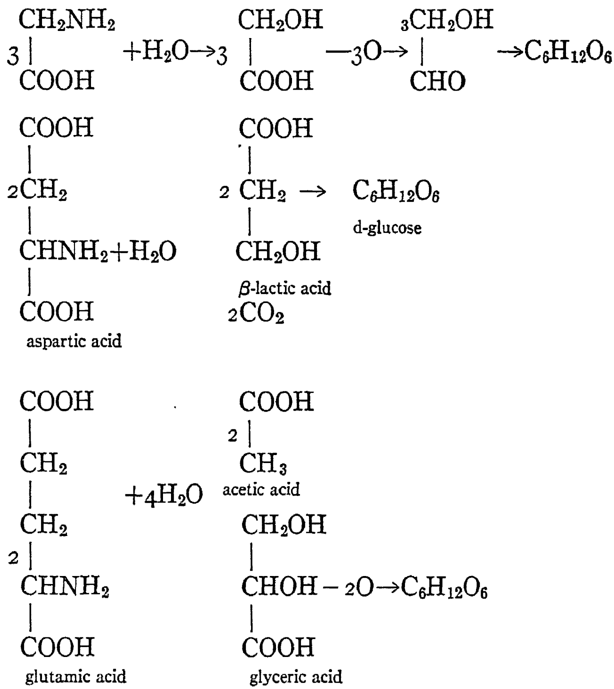
Carbohydrates may be formed from protein. Most of the work on these transformations has been done by animal-feeding experiments. The nitrogen free parts of glycocoll, alanine, aspartic acid, and glutamic acid containing 2, 3, 4, or 5 carbon atoms may be completely or partially transformed to d-glucose. All of the glycocoll and alanine may be so transformed, while three of the carbon atoms in aspartic and glutamic acids may be so converted.

The first step in the transformation of amino acid to carbohydrate is a hydrolytic deamination in which ammonia is split off and an hydroxyl group or a ketonic group added to the denitrogenized amino acid.

$$
\begin{array}{lllll}
CH_3 & & CH_3 & CH_3 & \\
| & & | & | & \\
2HCNH_2 & +O \rightarrow 2NH_3 & +2C=O & \rightarrow 2CO & \rightarrow C_6H_{12}O_6 \\
| & & | & | & \\
COOH & & COOH & CHO & \\
 & & \text{pyruvic acid} & \text{methyl glyoxal} &
\end{array}
$$

$$
\begin{array}{llll}
CH_3 & & CH_3 & \\
| & & | & \\
2CHNH_2 & +H_2O \rightarrow 2NH_3 & +2CHOH & \rightarrow C_6H_{12}O_6 \\
| & & | & \\
COOH & & COOH & \\
\text{alanine} & & \text{lactic acid} &
\end{array}
$$

Deamination is an important activity of cell life. Glycocoll by deamination then gives glycolic acid which on reduction yields glycolic aldehyde, three molecules of which may form one molecule of d-glucose.

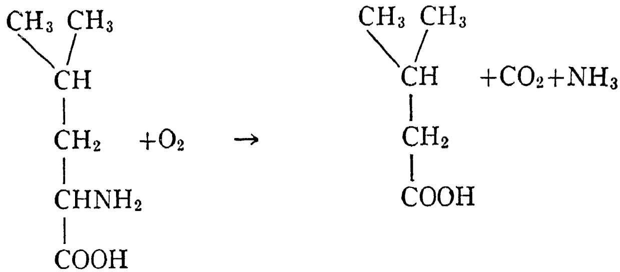


Serine, proline, ornithine, and arginine all may yield large amounts of sugars. Arginine is the only amino acid with more than five carbon atoms which yields glucose freely. Here the sugar probably comes from the ornithine part with five carbon atoms into which arginine may be converted by the enzyme arginase. Lysine is the only straight-chain amino acid which fails to yield sugar.

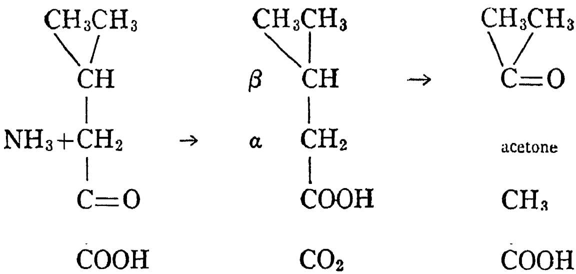
The production of carbohydrate from protein is not of as much importance in higher plants as in bacteria and animals. The tendency in plants is to stop the decomposition of protein at the stage of amides asparagine and glutamine. These amides are to be considered as the typical nitrogen residues from protein decomposition in plants. In animals the more common nitrogen residue is urea,

II. *Oxidative Deamination*

The first step in oxidative deamination of amino acids may be represented as follows: $RCH_2CHNH_2COOH + O_2 \rightarrow RCH_2COOH + CO_2 + NH_3$



leucine isovaleric acid

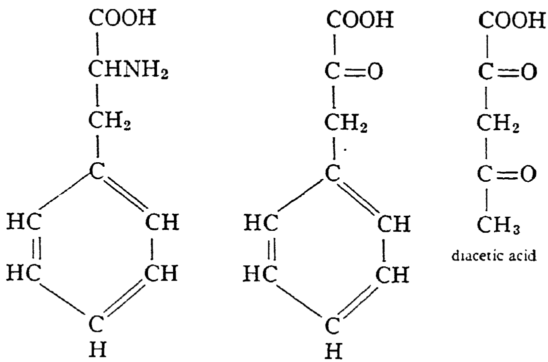


acetone

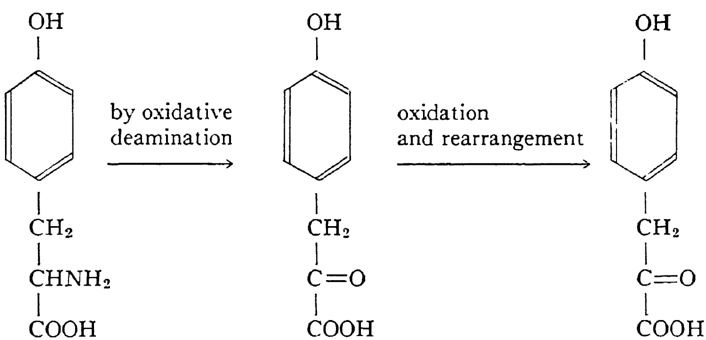
ketonic acid acetic acid

Tyrosine, phenylalanine, and tryptophane which contain benzene nuclei may be disintegrated in the organism to straight-chain compounds. On oxidative deamination phenylalanine is changed to phenylpyruvic acid, a compound which is probably of importance in respiration.

Phenols and compounds containing phenolic rings have been shown to be acceptors of oxygen. There is a group of phenoloxidases in plants, which are capable of inducing the oxidation of phenolic compounds. This indicates that the phenolic compounds may serve as carriers of oxygen and thus may stimulate respiration. The relation of tyrosine and other such cyclic amino acids to the formation of hydroquinone and related compounds is obvious.

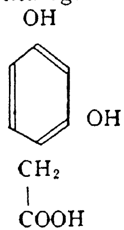


phenylalanine — phenylpyruvic acid — diacetic acid



tyrosine
p-oxyphenyl
α-aminopropionic acid → p-oxyphenyl pyruvic acid → hydroquinone pyruvic acid

By CO_2
cleavage



homogentisic acid

According to Lusk, some of the amino acids even when they are not completely oxidized may yield products of metabolism, either hydroxy or ketonic acids which act as stimulating substances to induce higher oxidation in the organism. In this way they may be of significance in controlling the rate or extent of cellular metabolism. The stimulating substances formed may be such things as phenylpyruvic acid.

III. *Origin of Amino Acids*

It seems common that glycocoll may originate from the cleavages of amino acids with longer carbon chains. The synthesis of glycocoll is evidently not difficult. The formation of amino acids from carbohydrates is not so well known as the reverse reaction. The close relationship between lactic acids and the carbohydrates on one hand and between lactic acid and alanine on the other hand suggests a ready means of amino acid synthesis from carbohydrate through the intermediate steps of lactic acid and ammonium pyruvate.

Alanine, phenylalanine, and tyrosine may be synthesized from ammonium salts of ketonic acids

$$\underset{\text{ammonium salt of ketonic acid}}{RCH_2COCOONH_4} \rightarrow \underset{\text{imino acid hydrate}}{RCH_2C{-}COOH}$$

$$\underset{\text{amino acid}}{RCH_2CHNH_2COOH}$$

IV. *Asparagin and Amino Acid Interconversion*

Asparagin in plants may be the source of nitrogen for complete protein synthesis. Sachs held the idea that protein could be formed from asparagin by the simple addition of fatty aldehydes. Loewi thought that in the presence of carbohydrates protein was formed from asparagin by a series of condensations. It is very probable that asparagin is an important intermediate in protein building. In plants it is a principal translocation form, being preferred over all other amino compounds. The distribution of asparagin in the cotyledons and hypocotyls of germinating lupine seed shows its importance as the translocation form of protein. In the axis asparagin represented 31.81% of the dry weight while in the cotyledons it represented 7.62%. In the cotyledons asparagin represented 22.7% to 26.2% of all of the amino acids; in the hypocotyl it represented 78.1% to 80.1% of all the amino acids. Evidently the amino acids produced by the cleavage of storage protein in the cotyledons are not translocated far as such, but are soon transformed to asparagin. Asparagin is found

abundant in plant parts in proportion to the abundance of leucine and glutamic acid. It probably originates from these amino acids, both of which are important cleavage products of the proteins, through oxidation to aspartic acid. For most amino acids the gradient of concentration in the germinating seed is from highest concentration in cotyledons to lowest concentration in the growing axis. For asparagin the gradient is exactly reversed, the highest concentration being in the axis and the lowest in the cotyledons. This indicates that asparagin is a secondary or possibly waste product produced after the primary protein cleavage into amino acids. Asparagin has been said to be the nitrogen waste of plants and comparable to the urea of animals.

Asparagin formation is favored by high oxygen concentration. This indicates that it is a product of protein oxidation. Asparagin may be easily demonstrated in the germinating seeds of legumes and also in many other seeds by the formation of marine blue crystals of copper asparaginate on the addition of copper acetate solution to sections of the tissue.

Under abnormal conditions for growth, such as in the exposure of seedlings to the air of laboratories containing ethylene and other gases, there is found a great accumulation of asparagin in the seedlings. Ethylene greatly increases the oxidation rate in tissues exposed to it in even low concentrations. Priamscharikow gives the following analysis of the asparagin content of seedlings grown in laboratory air.

	LABORATORY AIR		PURE AIR	
Days	*Cotyledons*	*Axis*	*Cotyledons*	*Axis*
5	.238	.445	.212	.231
10	.297	.524	.165	.217
15	.348	.625	.140	.289

V. *Proteins of Seeds*

The cereals have large amounts of prolamines which give much proline on hydrolysis, and large quantities of glutamic acid and ammonia. The proteins of wheats, rye, and barley contain but little lysine and not much arginine or histidine. The proteins of the legumes are relatively rich in lysine.

In general the proteins of a certain genera of plants may be very similar, but the proteins of the great groups of plants show wide variations in their constituents. The differences in the proteins stored in the seeds of different families of plants must certainly have a profound influence upon the development of the embryos which derive their protein from that stored by the parent plant. The development of animals is pro-

foundly influenced by the proteins contained in the diet, yet plants seem more able to synthesize amino acids so that the embryo should not be entirely dependent upon the storage protein of the seed. However, the nature of the chemical processes going on in the metabolism of the young plant will necessarily depend upon the kind of protein stored. The proteins of plants are highly specific, and, indeed, the genetic relationships between plants can be shown by serological reactions (Fig. 46).

VI. *Protein Storage in Seeds*

During the process of protein storage in seeds, asparagin is in greater abundance than any other amino acid. Other amino acids, particularly tyrosine and leucine, can be traced moving into the seed, but they are present in much smaller quantities than asparagin. On reaching the seed there seems to be a transformation of at least part of the asparagin into other amino acids, although in seeds there is usually found a high content of amide nitrogen. Nitrogen may move into the seed also in the form of nitrates and ammonium salts. It has been well established that there is a marked decrease of non-protein nitrogen in seeds during the process of ripening, with a simultaneous increase in protein nitrogen.

The condition in ripening seed of *Lupinus vulgaris* is a good example of this transformation. Protein nitrogen at the beginning of seed formation represented 0.204 gm. which later increased to 1.804 gms., while the non-protein nitrogen remained fairly constant in quantity. In *Lupinus angustifolius*, Wassiluff found in the unripe seed 30% non-protein nitrogen made up of 14% free amino acids, 9.8% asparagin, and 6.7% organic bases, while in the ripe seed there was no free amino acids, no asparagin, and only 5% of organic bases. In *Robinia pseudacacia* the unripe seed contained 25% non-protein nitrogen of which 20% represented amino acids, which on ripening of the seed were condensed to protein.

In a study of nitrogen transformations of pea seed, Zaleski showed clearly that the non-protein nitrogen is transformed into protein. He used the cotyledons of peas, determining the distribution of nitrogen in one-half as a control and analyzing the other half after storage for five days. This avoided the entrance of new materials through the placenta.

	Control	*Stored 5 days*
Protein nitrogen	79.2	89.2
Amino acid nitrogen	8.7	4.6
Organic base nitrogen	10.8	5.6
Other nitrogen compounds	1.4	.8

In ripening there is evidently an increase in protein nitrogen at the expense of all of the other forms. Eckerson in a study of the ripening of

wheat demonstrated the movement of non-protein nitrogen into the seed and came to the conclusion that the desiccation accompanying ripening was responsible for the formation of protein from other nitrogen compounds undergoing translocation into the seed.

In the process of protein formation in ripening seeds, practically all of the amino acids can be demonstrated. Evidently all of the building stones necessary for the formation of protein are synthesized in the seed from the substances translocated into it from the stem. The carbon chains of the amino acids are no doubt derived from carbohydrates. The phosphorus comes into the seed as phosphate. The nitrogen moves into the seed either as nitrate, ammonium salts, amides, or amino acids.

XXI. *Protein Catabolism*

The end-product of protein catabolism is generally ammonia. In this regard plants differ from animals in which the principal protein residue is urea. In plants urea has been found in fungi, especially as in the species of *Lycoperdon,* and in some quantities in flowering plants. In *Lycoperdon* urea may represent from 1 to 10% of the dry weight. Urea is produced from arginine by the action of arginase.

$$NH{=}C(NH_2)NH{-}CH_2{-}CH_2{-}CH_2{-}CH(NH_2){-}COOH + H_2O \rightarrow$$

$$\underbrace{NH_2{-}CONH_2}_{\text{urea}} + \underbrace{NH_2{-}CH_2{-}CH_2{-}CH_2{-}CH(NH_2){-}COOH}_{\text{ornithine}}$$

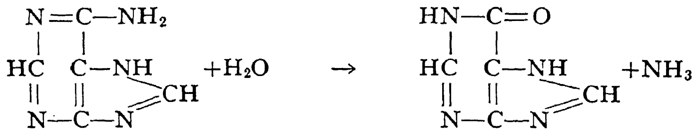
Arginase is present generally in plants. Urea is also produced from other amino acids. Urea is decomposed by the enzyme urease, with the production of ammonia and CO_2.

$$\underbrace{\begin{matrix} NH_2 \\ NH_2 \end{matrix}\!\!>C{=}O}_{\text{urea}} + 4H_2O \rightarrow 2NH_4OH + H_2CO_3$$

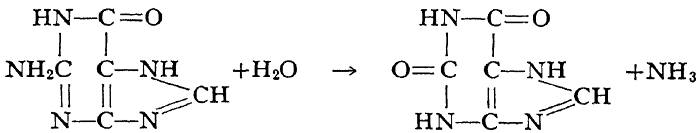
Urease is almost universally distributed in plants, so that if urea should be produced in plants as it is in the catabolic process of animals, the urea would not accumulate to high concentrations on account of its decomposition to ammonia by urease. Urease has been produced in crystalline form from the jack-bean.

The ammonia produced by protein catabolism is used again for the synthesis of amino acids, using the energy and carbon chains derived from carbohydrates for the resynthesis.

A number of the simpler nitrogen bases such as the alkaloids, especially those of the caffeine series, caffeine, theine, and theobromine, and other compounds related in structure, may be products of protein catabolism. The adenine and guanidine groups of the nucleic acids under the action of the oxidoreductase enzyme, xanthoxydase, split off ammonia and are transformed into xanthine bases.



adenine hypoxanthine

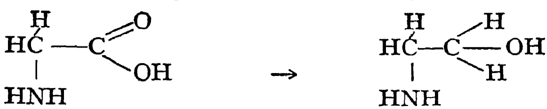


guanidine xanthine

Theobromine is 3, 7-dimethylxanthine. Theine is 1, 3-dimethylxanthine, and caffeine is 1, 3, 7-trimethylxanthine.

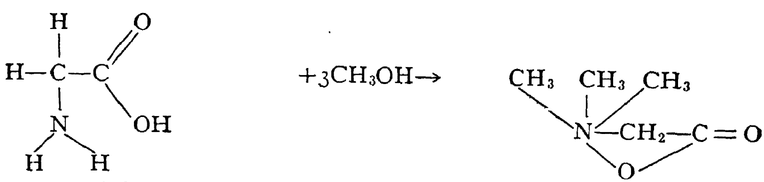
Generally in plants the amino acid residues are again built up into amino acids whenever carbohydrates are available for the resynthesis. But under the action of bacteria and fungi and under abnormal conditions of metabolism, part of the amino acid may produce basic nitrogen compounds.

Glycine and serine may give rise to amino-ethyl alcohol.



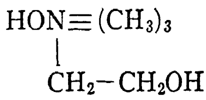
glycine amino-ethyl alcohol

Glycine may give rise also to betaine on methylation.

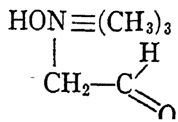


glycine betaine

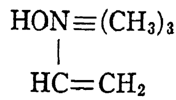
Choline may be produced from amino-ethyl alcohol; and its aldehyde, muscarine, may be similarly produced. Probably neurine is derived from



choline



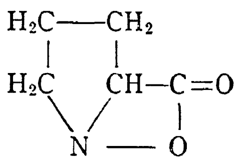
muscarine



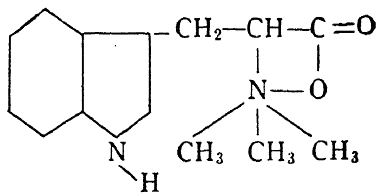
neurine

Choline is a constituent of all plants, and is non-poisonous. Muscarine is found in some fungi, particularly in the poisonous *Amanita muscaria*. Neurine may be produced by bacterial action and is particularly abundant in decomposing brain tissue.

In similar manner under conditions favoring methylation, other amino acids produce compounds related to these substances. Proline gives rise to stachydrin, and tryptophane may produce hypaphorin.



stachydrin



hypaphorin

Leucine may produce isoamylamine.

$$(CH_3)_2{=}CH{-}CH_2{-}CH(NH_2){-}COOH \rightarrow (CH_3)_2{=}CH{-}CH_2{-}NH_2 + CO_2$$

leucine

Valine may produce isobutylamine.

$$\underset{\text{valine}}{(CH_3)_2{=}CH{=}CH(NH_2){-}COOH} \rightarrow \underset{\text{isobutylamine}}{(CH_3)_2{=}CH{-}CH_2NH_2}$$

These compounds are of especial interest because they are highly poisonous and may be produced in foods by bacterial action, especially under anaërobic conditions.

PART V

PART V

PHOTOSYNTHESIS

CHAPTER XXII

MATERIAL EXCHANGE IN PHOTOSYNTHESIS

The major part of the reduction of carbon in the organic world takes place through photosynthesis, which is dependent upon the absorption of light by chlorophyl. The reduction of carbon by the use of energy liberated in the oxidation of such reduced substances as iron or sulphur in chemosynthetic processes, although it may have been of great importance in early stages of evolution and still is of importance in the metabolism of bacteria, yet does not lead to the production of such large quantities of organic substances as are produced in photosynthesis by green plants. Possibly other pigments than just chlorophyl take part in photosynthesis. Bacteriopurpurin has been stated to be a photosynthetic pigment in the purple sulphur bacteria which are devoid of chlorophyl. It is not just certain that other pigments such as carotinoids may not function in photosynthetic processes. But the principal process for the storage of the energy of light as chemical energy for the use of plants and animals is the photosynthesis of plants which contain chlorophyl.

I. *Definition of Photosynthesis*

Photosynthesis may be defined as the process whereby plants absorb carbon in the fully oxidized condition and reduce it by the absorption of light energy. The use of this name for the process is to be preferred to the term carbon assimilation, for the latter may not require the absorption of light. Chemosynthetic processes occur in plants which lead to carbon assimilation, yet these are not photosynthetic processes. Under the term *photosynthesis* should be included all reductions of carbon in which light energy is stored in plants, whether the energy absorption and photocatalytic action are due to the presence of chlorophyls or of other substances which may absorb light or take part in the process. Photosynthesis may be considered as an especial photocatalysis occurring in plants. If the reduction of carbon dioxide or carbonates should be effected outside of the plant by the use of light energy, it would be preferable to use the more general term *photocatalysis* for such processes.

The photosynthetic process involves the action of physical processes, such as the diffusion and solution of carbon dioxide and the products of the reaction, and also chemical processes. Part of these processes may be carried on without light, heat may be absorbed in some reactions, but certainly the energy of light must be introduced before there is any considerable transformation of radiant into chemical energy. Heat alone does not produce photosynthesis; evidently the photocatalysis is confined to the wave-lengths of radiant energy which are visible as light. Blackman found that photosynthesis shows two separate phases; one stage is dependent upon light, the other, the Blackman reaction, can proceed in the dark. Probably each stage involves several individual physical or chemical processes.

II. *Source of Carbon*

The source of carbon for photosynthesis is generally the bicarbonate HCO_3^- ion although we are accustomed to refer to the carbon dioxide of the atmosphere as the source. The atmospheric CO_2 is produced from the respiration of plants and animals, by the combustion of plant remains as wood or coal, and by the solution of carbonate rocks. To get into the cell, the carbon dioxide must go into solution in the various aqueous phases of the protoplast. The acidity in the protoplast is such that bicarbonate HCO_3^- is present. If the alkalinity becomes considerable, calcium carbonate will deposit in the cells as a little-soluble precipitate. In high cell acidity free carbonic acid may be present. In water plants the absorption is mainly from solution, and in the usual water supply the bicarbonate will be most abundant. Calcium is usually present in sufficient quantity to precipitate the normal carbonate if the solution becomes slightly alkaline (pH 8). (See Graph, Fig. 11.) The acidity usually found in the water of lakes and streams is such that calcium and other cations are present as the bicarbonates. Acid waters may contain free carbonic acid, H_2CO_3. The production of normal carbonate in the water by the removal of bicarbonate ions in photosynthesis frequently causes the precipitation of calcium carbonate as an insoluble incrustation over the surface of water plants. This can be seen on the surface of *Chara* and *Ceratophyllum*.

The concentration of carbonate ions available to the plant is determined by the acidity, whether entrance through the cell membrane is as dissociated ion HCO_3^- or $CO_3^{=}$ or undissociated H_2CO_3.

III. *Diffusion of CO_2 into the Plant*

The entrance of bicarbonates into the algæ and other water plants is relatively simple diffusion through the whole surface of the filament,

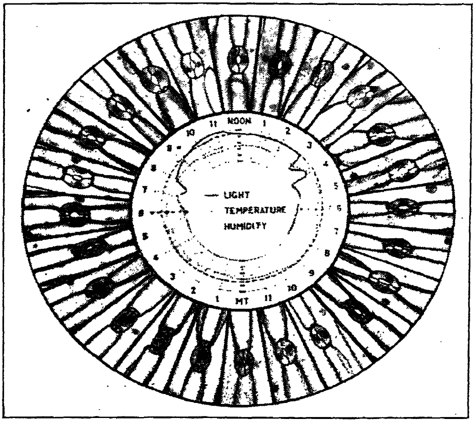


FIG. 53.—Stomata of onion, × 240, and factors during a 24-hour day. (After Loftfield.)

leaf, or stem. The fully imbibed cellulose wall is easily permeable to carbonates. In land plants the only parts in contact with carbonates in solution are the roots. The entrance of carbonates into the plant from the soil is of little or no importance for the photosynthetic process. The principal absorption is by the leaves and stems. Stems which carry on photosynthesis are provided with openings for gas exchange. There are frequently long continuous passages for gases in stems. The structure of leaves is generally such as to provide for gaseous exchange with the atmosphere. The leaf is arranged with large interconnecting intercellular spaces providing for free gaseous diffusion. The leaf surface may be covered with enormous numbers of small openings called *stomata*, which open directly into the intercellular spaces within the leaf. Stomata may be present on both sides of the leaf, but they are usually more abundant on the surface away from the sun's exposure. In some plants stomata may be found on the under side of leaves only. The open stomata provide for the entrance and exit of gases, especially the entrance and exit of CO_2, O_2, and water vapor. The entrance and exit of these gases are controlled to a great degree

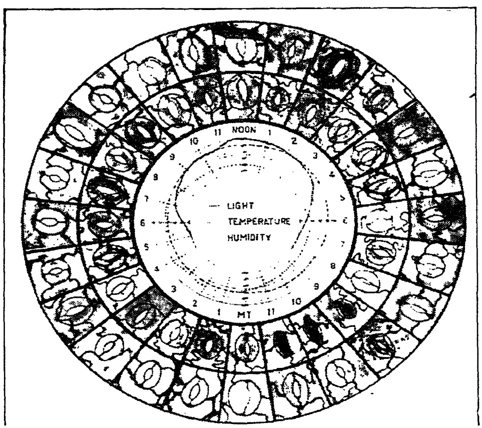


FIG. 54—Condition of upper (outer) and lower (inner) stomata of potato, × 240, at each hour of a 24-hour day, together with curves for sunlight, temperature, and humidity. (After Loftfield

by the opening or closing of the stomatal pores. This is especially concerned with the regulation of water loss from the leaf by transpiration. The daily march of the stomatal opening and its relation to temperature, light, and humidity are shown in Figs. 53 and 54. The function of the movable guard cells of the stomata is to prevent excessive evaporation into an unsaturated atmosphere or the attendant desiccation of the leaf cells. The closing of stomata to prevent water loss also stops the direct diffusion of CO_2 and O_2 through them. The stomatal regulation is a necessary evil as far as the photosynthetic process is concerned. On closing these direct paths for gaseous diffusion, photosynthesis is interfered with, owing to the slower rate of diffusion of CO_2 and O_2 through the cuticle. The absorption of CO_2 through the cuticle may be so slow as to limit the photosynthesis. Practically all of the CO_2 for photosynthesis enters through the stomata. The relative amount of carbon dioxide taken in by the upper and lower surfaces of leaves is practically proportional to the number of stomata on the two sides (Table 19).

TABLE 19

ASSIMILATION OF CARBON DIOXIDE BY THE TWO SURFACES OF LEAVES
(Data from Brown and Escombe)

Species	*Time in hours*	*Leaf area in sq. cm.*	*CO_2 assimilated in c. c.* Upper surface	Lower surface	*Stomatic ratio Upper: Lower*	*Assimilation ratio Upper: Lower*
Colchicum speciosum..	5.75	59.44	4.34	3.26	100 : 119	100 : 72
Senecio macrophyllus..	4.75	28.27	3.90	3.60	100 : 126	100 : 92
Senecio macrophyllus..	4.25	28.27	5.80	4.20	100 : 126	100 : 72
Rumex alpinus.......	5.0	59.44	5.70	8.90	100 : 269	100 : 144
Rumex alpinus.......	5.5	59.44	7.50	9.81	100 : 269	100 : 130
Nuphar advenum.....	2.0	76.97	2.20	0.00	100 : 0	100 : 0
Catalpa bignonioides..	1.85	79.03	0.00	4.91	0 : 100	0 : 100
Catalpa bignonioides..	2.3	79.03	0.00	8.96	0 : 100	0 : 100

The stomata on the illuminated upper side of leaves may be wider open than those on the under side. Blocking of the stomata by wax effectively cuts off the diffusion of gas through them.

The absorption of CO_2 from the intercellular spaces of the leaf is possible for nearly all cells. But the entrance to the intercellular spaces from the atmosphere takes place through the comparatively small fraction of the leaf area represented by the sum of the areas of the stomatal pores. In *Catalpa bignonioides*, Brown and Escombe found that the

stomatal pores represent only 0.9% of the whole leaf surface. This leaf absorbed from the atmosphere containing .03% of CO_2, 0.07 c. c. of CO_2 per sq. cm. of leaf surface per hour in its photosynthesis. The diffusion rate of the CO_2 must take place at the rate of 7.77 c. c. per sq. cm. per hour, since it all passes through the stomatal pore. But n/1 NaOH solution absorbs from the same atmospheric concentration of CO_2 (.03%) under the same conditions only 0.12 c. c. of CO_2 per sq. cm. of absorbing surface per hour. The rate of absorption through the stomatal pore must be about fifty times as fast as the absorption of CO_2 by the surface of n/1 NaOH.

This greatly increased rate of diffusion through pores is found also for small openings in diaphragms of metal, so that it is not a special property of stomata but results from the mechanics of gaseous diffusion and the paths followed by molecules in the gaseous condition in free diffusion. In free diffusion of gas molecules through a large aperture, the lines of flow of the molecules will be as pictured in section A of Fig. 55. The concentration of the carbon dioxide will increase from zero at the surface of the disk to its maximum concentration theoretically at infinite distance from the absorbing surface. If the aperture is large, carbon dioxide will diffuse equally rapidly toward the surface and the path of the gas molecules will be represented as straight lines perpendicular to the absorbing surface. In this case we are concerned with gaseous molecules diffusing in straight lines, and the quantity passing through the aperture will be proportional to the area of the aperture

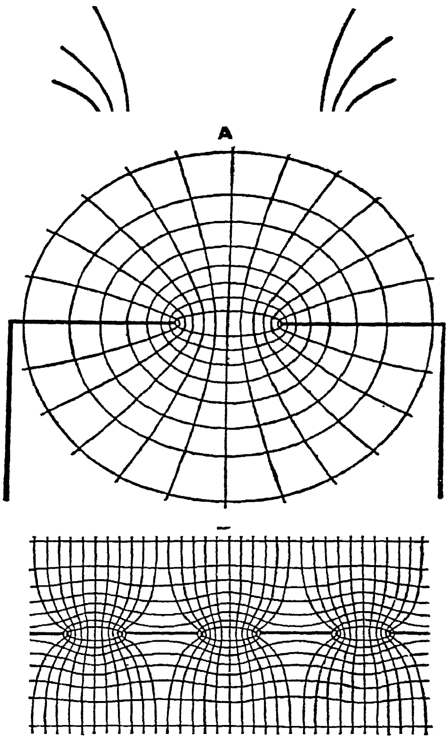


FIG. 55.—A and B. Paths of diffusion of gas molecules through openings. C. Brown and Escombe's conception of the lines of flux in a diffusive column through septa.

when other conditions such as the concentration gradient remain the same.

In accordance with Fick's law,

$$dQ = -DA\frac{dC}{dx}dt$$

the quantity, dQ, of a substance diffusing through an opening is proportional to the cross-sectional area, A, the time, dt, and the gradient of concentration between the two sides, $\frac{dC}{dx}$, multiplied by the diffusion constant, D, of the gas.

At the edges of the aperture, gas molecules can move in at an angle, the paths forming a series of parabolic curves. But this movement at the margin is only a relatively small part of the total. The marginal absorption is a small fraction of the total absorption when the aperture is large. But as the aperture is made smaller, the marginal absorption becomes greater as shown by the following table (Table 20).

TABLE 20

DIFFUSION OF CARBON DIOXIDE THROUGH APERTURES OF VARIOUS SIZES
(Data from Brown and Escombe)

Diameter of aperture in mm.	*CO_2 diffused per hour*	*CO_2 diffused per sq. cm. per hour*	*Relative areas of apertures*	*Relative diameters of apertures*	*Relative amounts of CO_2 diffused in unit time*
22.7	0.2380	0.0588	1.00	1.00	1.00
12.06	0.0928	0.0812	0.28	0.53	0.39
12.06	0.1018	0.0891	0.28	0.53	0.42
6.03	0.06252	0.2186	0.07	0.26	0.26
5.86	0.05558	0.2074	0.066	0.25	0.23
3.233	0.03988	0.4855	0.023	0.14	0.16
3.216	0.03971	0.4852	0.020	0.14	0.16
2.117	0.02608	0.8253	0.008	0.093	0.10
2.00	0.02397	0.7629	0.007	0.088	0.10

For apertures of the dimensions of stomata the total diffusion becomes proportional to the perimeter of the aperture and not proportional to its area, because the lines of diffusion are those represented in section B of Fig. 55. The diffusion in a straight line toward the absorbing surface is a relatively small part of the total diffusion. The marginal diffusion represents practically the total, and the rate of absorption is proportional to the dimension of the margin, or perimeter. In the larger aperture, as

shown in A, the absorption is proportional to the area of the aperture. Stomata are seldom circular in outline. They are more frequently elliptical when fully open. When partly closed, they approach the dimensions of a slit, because the closure is due to the change in position of two guard cells. For apertures of such shape, the diffusion is more nearly proportional to the perimeter than to the area.

In the case of stomata with large hypostomatal cavities, the diffusion lines are as shown in section B of Fig. 55 in still air. There is an apparent crowding of the diffusion lines through the small opening. When the air outside of the leaf is in motion, as it usually is, the concentration shells outside disappear owing to the rapid renewal of the external molecules of the air current. In the case of depressed stomata, the length of the stomatal tube must be taken into account, for through this tube the diffusion will be in straight lines. If the stomata are very close together, there will be an overlapping of the diffusion lines. When the pores are a few diameters apart, there is practically no interference of the gas molecules, as shown in section C of Fig. 55. Each pore then acts practically independent of the others. The stomata of leaves are usually eight diameters or more apart, so that diffusion through each is nearly at the maximum efficiency. Actually, the stomata when they are fully open allow more diffusion than is necessary to account for the photosynthetic rates shown by leaves. The partial closure of the stomata does not greatly affect the perimeter and, consequently, does not alter the absorption rate greatly. The throttling action becomes evident when there is complete or almost complete closure.

With the heating and cooling of the leaf and the air confined within it, there is mass streaming of gases. The temperature of the leaf fluctuates with the atmospheric temperature and with the heating effect of sunlight exposure which is exceedingly variable. The passage of every cloud obscuring the sun causes changes in the leaf temperature and expansion or contraction of the air contained in it, resulting in mass movements of gases through the stomata.

The diffusion of both carbon dioxide and oxygen into the cells of the leaf through the cellulose walls is in solution in the water present in the wall. If the wall is desiccated, the diffusion is decreased. The cuticle of plants contains cutin and suberin, which are fatty in nature. The diffusion of water from the epidermal cells is very little, but cutin and suberin deposits in cellulose favor the passage of CO_2 and O_2 through the epidermal layer. However, the diffusion through the upper cuticle of leaves which have stomata only on the lower side represents only about 3% of the diffusion through the open stomata and cuticle of the lower side of the hypostomatous leaf.

IV. *Evolution of Oxygen*

Equally as important as the absorption of carbon dioxide in photosynthesis is the production and evolution of oxygen. For the diffusion of oxygen the same conditions will hold as found for CO_2. The liberation of free oxygen must occur under high oxygen tension within the cells, probably at full or supersaturation of the cell fluids. The presence of free oxygen should tend to decrease the rate of the photosynthetic processes by the mass action of this product of the reaction. The photosynthetic reactions are usually written so as to indicate the evolution of molecular oxygen. Nascent or atomic oxygen is a very strong oxidizing agent. There must be a relatively enormous change in the oxidation

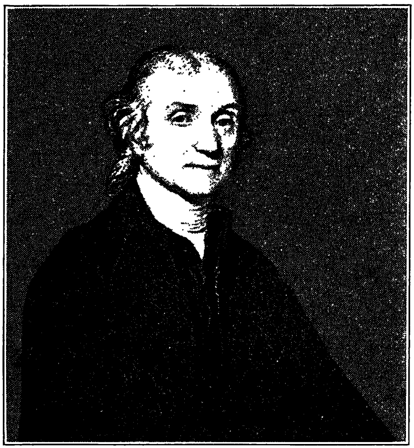


FIG. 56.—Joseph Priestley, 1733–1804.

"Plants instead of affecting the air in the same manner with animal respiration, reverse the effects of breathing, and tend to keep the atmosphere pure and wholesome, when it is become noxious in consequence of animals either living, or breathing, or dying, and putrefying in it."
Experiments and Observations on Different Kinds of Air. London, 1775.

potential within photosynthesizing cells upon exposure to light. The oxygen is given off to the air as molecular oxygen. If the oxygen evolved is in molecular condition at the moment of reduction of the carbon, the oxidation potential in the cell should be somewhat reduced from that of atomic oxygen, since considerable energy is evolved in the formation of molecular from atomic or nascent oxygen.

It was in an experiment upon the purification of the air by plants that Priestley (Fig. 56) entered upon the series of experiments which led to his discovery of vital air or oxygen (1770). He found that sprigs of mint

placed under a bell-jar containing an atmosphere which had become vitiated by animal respiration were able to purify the air so that animals could again live in it. The oxygen evolution by plants, although observed first by Priestley, could not be repeated by him. It remained for Ingenhousz in 1779 to show that the factor which Priestley had not taken into account was the light exposure of the plants. Ingenhousz showed also that only the green parts of plants possess the power of purifying the air from the products of the respiration of animals. Senebier (Fig. 57)

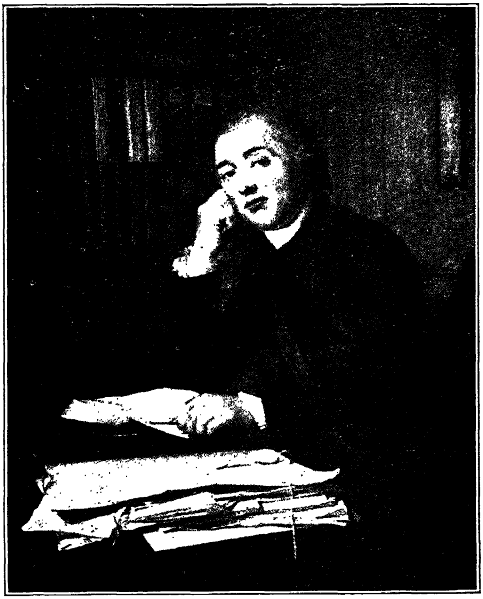


FIG. 57.—Jean Senebier, 1742–1809. (From a portrait in the collection of Dr. J. C. Bay.)

"Then if the fixed air (CO_2) dissolved in the water of the atmosphere within the parenchyma combines with the light and all the other elements of the plant, if the phlogiston of that fixed air (CO_2) is actually precipitated in the organs of the plant, if this precipitate remains as it seems since that fixed air comes out of the plants in the form of dephlogisticated air (O_2), it is clear that fixed air (CO_2) combined in the plant with the light, leaves there a substance which had not been there, and my experiments on etiolation suffice to demonstrate this."

Memoirs physico-chymiques. Geneva, 1782.

first suggested in 1783 that the evolution of oxygen (vital air) by plants in the sunlight is accompanied by the absorption of carbon dioxide (fixed air). N. T. de Saussure (Fig. 58), by quantitative methods of gas analysis, excellent for his time, showed the relationship between the quantities of carbon dioxide absorbed and the oxygen evolved by the green plant in

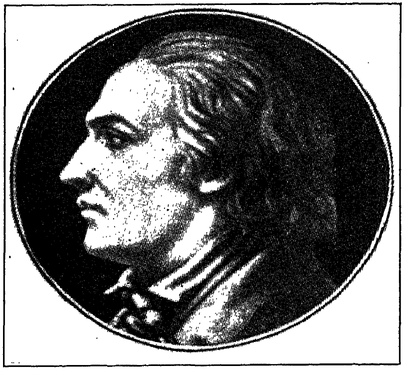


FIG. 58.—Nicolas Théodore de Saussure, 1767–1845. (Picture loaned by Dr. J. C. Bay.)

"The roots of plants take up the salts and the extracts, but in smaller proportions than the water which contains these salts and extracts in solution.

"A growing plant does not take up all the substances contained in a solution in the same proportions; it takes certain exceptions among them; in general, it takes in greatest amount the substances whose solutions are least viscous.

"When the plants build into their structure the oxygen and hydrogen of water, this loses its liquid properties. This assimilation occurs to an observable extent only when the plants at the same time assimilate carbon.

"The water, bound or transformed into solid substance by the plant, evidently can lose its oxygen in the form of a gas only after the death of the plant or one of its parts.

"If the plants, which assimilate the oxygen and hydrogen of water, out of free contact with oxygen begin to ferment, then they produce carbonic acid gas from their own substance. The oxygen of the combined water can combine with the carbon for the formation of carbonic acid gas; and the plants or the vegetating parts give off indirectly one of the constituents of the original water in that they release the oxygen of the latter.

"But in no case do the plants directly decompose the water by assimilating its hydrogen and giving off its oxygen in the form of gas; they exhale oxygen gas only with the coincident decomposition of carbonic acid gas."

"I allowed the roots of several peppermints (*Mentha piperita*) to dip into flasks filled with distilled water, and let the plants grow in the sun on a flower bench protected from rain, outside of a window. Since I took up some of these plants at the same time and at the same place and dried them, I proved to myself that 100 parts by weight of them which I wished to let grow in distilled water contained 40.29 parts of dry substance, from which after heating there was left 10.06 parts of coal.

"One hundred parts by weight of peppermint after growth for two and one-half months in free air weighed green 216 parts, but this increase in weight tells nothing since it is perhaps to be ascribed to the increase of the water of growth, which continually increases in the plants if they are planted in a place more moist than that on which they previously grew. Through drying at air temperature they went back to 62 parts by weight.

"By the use of air and water the plants accordingly increased their dry substance by 21.71 parts. These 62 parts on heating produced 15.78 parts of coal or 4.82 parts more than they would have produced if they had not grown in distilled water. When I allowed the same plants under the same conditions to grow in a weakly lighted place, I found that they had lost a small part of their carbon content.

"If green plants are exposed in atmospheric air to the alternating action of day and night, they breathe alternately in and out oxygen mixed with carbon acid gas.

"The Oxygen breathed in by green plants is not assimilated by them; it is on inspiration changed to carbonic acid gas."

Recherches chimiques sur la végétation. Paris, 1804.

sunlight (1804). He showed also that an increase in weight accompanied photosynthesis, and that through the absorption of carbon dioxide of the air there was an increase in the carbon content and dry weight of the plant.

V. *Water Used in Photosynthesis*

That the elements of water, H and O, are assimilated in photosynthesis was proved by De Saussure. In the formation of carbohydrates the elements hydrogen and oxygen are introduced in the same proportions in which they exist in water. This suggests that water and carbon dioxide, or H_2CO_3, are the principal reacting substances. Photosynthesis is just as much an assimilation of hydrogen and oxygen as of carbon. It is evident from this that water is required for photosynthesis.

CHAPTER XXIII

LEAF PIGMENTS

I. *Chloroplasts*

The chlorophyl of higher plants is contained in special organs of the cell, the chloroplasts. The chloroplasts of higher plants are mainly lens-shaped bodies which may be moved with the cytoplasm. In lower plants the chloroplasts may be of various shapes and sizes. In the blue-green algæ the pigment evidently is distributed throughout the cell and is not aggregated into special structures.

Chloroplasts may be solid or fluid, depending upon the consistency of the protein-containing stroma. Most chloroplasts are fairly firm gels, but the RHODOPHYCEÆ have fluid chloroplasts. There may be visible differences in the distribution of chlorophyl through the stroma. In *Selaginella* the pigment is found mostly at the surface of the plastid. Several other plants show dark-green shells of chlorophyl at the surface of the chloroplast.

Chloroplasts usually arise from the division of chloroplasts. Cells which have lost their chloroplasts may not reform them, and albino cells may be produced by their progeny. This lack of regeneration of chloroplasts in albino cells led to the fantastic idea that chloroplasts are separate individually from the plant cells and live symbiotically with them. In animals cases are known where the chloroplasts of plant cells are captivated and held symbiotically by the animal in a functioning condition for the production of carbohydrates. For instance, *Chlorella* lives symbiotically with *Hydra*. The *Chlorella* cytoplasm and nucleus may be destroyed, leaving only the chloroplasts which live symbiotically with the cells of the hydra for some time.

In some cases chloroplasts seem to come from mitochondrial structures which are too small to identify as chloroplasts, as in pea (*Pisum sativum*) and asparagus (*Asparagus officinalis*) stems. In these cases it is doubtful whether there is transformation of the mitochondria into chloroplasts; at least not all cells containing mitochondria develop chloroplasts. One can assume that an exceedingly small chloroplast already existed in the mitochondrium. In parts of plants removed from the light, chlorophyl does not develop in the plastid. This may be seen in

the tubers of potato. These plastids function for starch deposition and are true leucoplasts. When they are exposed to sunlight, however, they may again function as chloroplasts and will form chlorophyl.

It seems that the chlorophyl pigments are the key in the photosynthetic processes, because the plastid does not form carbohydrate until after the chlorophyl appears, although yellow pigments may be present. The protoplasm of the stroma may perform a major function in photosynthesis, for a mild disturbance of the stroma, such as a decrease of water, will upset the functioning of the photosynthetic processes.

II. *Chlorophyl Formation*

In many cases chlorophyl may be formed in cotyledons of seeds or in the inner bark without apparent light exposure at any high intensity. Thus the seeds of pines and ginkgo regularly may form green pigments within the cotyledons. Lemons frequently show green-colored cotyledons within the fruits, and the inner testa of cucurbits has green pigments. Probably some light may reach these structures at some stage in their formation. The general conditions for chlorophyl formation require the presence of light as a catalyst, yet the intensity of the light required can be very low.

Chlorophyl can be formed in the dark by some leaves if they are supplied with such sugars as sucrose, maltose, glucose, fructose, or raffinose, or with glycerin.

III. *Relative Abundance of Chloroplast Pigments*

The ratio of the pigment constituents does not change much in higher plants.

QUANTITIES OF GREEN AND YELLOW PIGMENTS PRESENT IN LEAVES

(Data from Willstätter and Stoll)

Pigment	*Parts per thousand of fresh leaves*	*Parts per thousand of dry weight*
Chlorophyl-a	2.0	6.3
Chlorophyl-b	0.75	2.4
Carotin	0.17	0.5
Xanthophyl	0.33	0.9

In the green algæ, chlorophyl-b is relatively more abundant and also there are more yellow pigments.

QUANTITIES OF GREEN AND YELLOW PIGMENTS PRESENT IN ULVA LACTUCA

(Data from Willstätter and Stoll)

Pigment	*Parts per thousand of fresh leaves*	*Parts per thousand of dry weight*
Chlorophyl-a	0.165	0.936
Chlorophyl-b	0.117	0.666
Carotin	0.024	0.138
Xanthophyl	0.064	0.365

In the brown algæ the proportions are variable, and there is present also the characteristic pigment of this group, fucoxanthine.

The usual ratio of the chlorophyl constituents in higher plants is as follows:

chlorophyl-a : b = 3:1
chlorophyls : carotinoids = 3:1
carotin: xanthophyl = 1:2

In shade leaves the ratio of chlorophyls to carotinoids is increased. The ratio in such leaves is from 6 to 7. Chlorophyl-a is more abundant in shade leaves in its proportion to chlorophyl-b.

IV. *Discovery and Separation of the Leaf Pigments*

The pigments of the green leaf were extracted and given the name *chlorophyl* by Pelletier and Caventou in 1818. The definite separation of the components of the green-colored extract from leaves was made by Stokes (Fig. 59) in 1864. He used the method of partition of the pigments between immiscible solvents which had first shown to Frémy in 1860 that yellow pigments were present along with the green. Stokes observed the differences in the spectroscopic absorption by the components of the leaf extract and came to the conclusion that there were two green and two yellow pigments in leaves, but his work was disregarded. The separation of the yellow pigments from the green chlorophyls was further perfected by Frémy. Later (1871) Timiriazev gave the name *xanthophyl* to the extracted yellow pigment. The separation of the leaf pigments by means of benzine and alcohol was introduced by Kraus in 1872. In the separation of the leaf extract by Kraus's method, Dipple in 1878, using the spectroscopic method, observed that there was present a yellow pigment with properties different from xanthophyl, and that there were two yellow pigments in leaves. Borodin (Fig. 60) in 1883 actually crystallized the two yellow pigments and described their solubility differences. The work of Stokes was largely overlooked, and required the confirmation of later workers before it was accepted by physiologists. It was commonly

held that there was only one green pigment in the leaf. Tswett (1906), by the introduction of adsorption methods, contributed largely to the clearing up of the confusion of statements regarding the leaf pigments. By percolating the leaf extract through a column of fine calcium carbonate, he found that the different pigments could be separated from the different layers of the column on which they were adsorbed. Tswett confirmed the statement of Stokes that two green pigments were present. Also, he separated from deeply yellow daffodils and narcissus five yellow pigments which he named collectively *carotinoids*. These were four xanthophyls varying in their adsorption characteristics and carotin which was not adsorbed at all by the calcium carbonate.

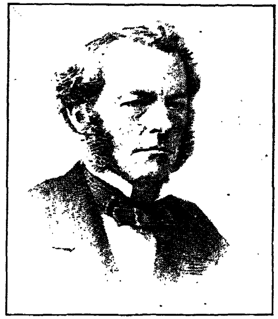


FIG. 59.—Sir George Gabriel Stokes, 1819–1903.

"I find the chlorophyll of land-plants to be a mixture of four substances, two green and two yellow, all possessing highly distinctive optical properties. The green substances yield solutions exhibiting a strong red fluorescence; the yellow substances do not. The four substances are soluble in the same solvents, and three of them are extremely easily decomposed by acids and even acid salts such as bisoxalate of potash; but by proper treatment each may be obtained in a state of very approximate isolation, so far at least as coloured substances are concerned."

On the Supposed Identity of Biliverdin with Chlorophyll, with Remarks on the Constitution of Chlorophyll. Proc. Roy. Soc., 13: 144 1864.

"For convenience and rapidity of manipulation, especially in the examination of very minute quantities, there is no method of separation equal to that of partition between solvents which separate after agitation. . . . Bisulphide of carbon in conjunction with alcohol enabled the lecturer to disentangle the coloured substances which are mixed together in the green colouring-matter of leaves."

On the Application of the Optical Properties of Bodies to the Detection and Discrimination of Organic Substances. Journ. Chem. Soc., 17; 304, 311. 1864.

The most important contributions to the knowledge of the properties and chemical constitution of the chlorophyls has been made by Willstätter and his students. This will be discussed in detail in a later paragraph.

FIG. 60.—Ivan Parfiniewicz Borodin.

"If one touches gently microscopic sections of green leaves of various plants with alcohol and allows the preparation to dry out slowly under the cover-glass, there often appear highly characteristic green crystals."

Ueber Chlorophyllkrystalle. Botanische Zeitung, 40: 608–609, 621–626. 1882.

In the preparation of chlorophyls from the green leaf we find that solvents which dissolve the purified pigments do not extract them from dry leaves. A few drops of water increase the solubility of the pigments in petrol ether. The addition of water may result in changes in the state of the chlorophyls whereby

they become soluble. Chlorophyls probably are present in solution in lipoids, and these may be distributed in droplets in the colloidal condition. There is also spectroscopic evidence that the chlorophyls in the leaf are present in the colloidal condition, for there the absorption lines are shifted toward the red end of the spectrum from the position shown by the pure pigments in true solution.

The chlorophyls of some leaves are easily obtained in crystalline form. Borodin found 190 out of 770 species examined which showed a good formation of crystals of chlorophyls when sections were moistened with alcohol and the solution was allowed to evaporate slowly. Leaves of 200 species gave only amorphous chlorophyl masses. Willstätter and Oppe explain the difference between crystalline and the amorphous chlorophyls as being due to the action of chlorophyllase, an enzyme resembling esterase. When amorphous chlorophyls are put into contact with alcohol and with some plant tissue in which chlorophyllase is abundant, they are converted into cystalline chlorophyls. The esterase, chlorophyllase, hydrolyzes the phytyl chlorophyllides and produces the free acids, which may then be esterified under the action of the esterase to the ethyl or methyl chlorophyllides according to the alcohol present. The ethyl and methyl chlorophyllides are easily crystallizable. Their presence accounts for the tendency for the production of crystalline chlorophyls from certain leaves. Slow extraction under conditions such that the chlorophyllase is not destroyed will account for high yields of crystalline chlorophyls. To avoid saponification and esterification it is necessary only to use solvents which do not permit of this action, such as acetone.

In the extraction of chlorophyls from the chloroplast there is first an action of the solvent upon the plastid. The stroma of the plastid is a hydrophyllous colloid which will swell in water. The swelling of the plastid favors the taking of the chlorophyl from the stroma. Liebalt found chloroplasts swelling enormously in water. The stroma of the chloroplast is protein in nature and is not soluble in fat solvents, but it may be dehydrated or agglutinated by alcohol or acetone. Under the action of these solvents various reticulate structures appear in the stroma, and these have often been figured by cytologists as showing the structure of chloroplasts. The green pigments of the chloroplast are soluble in lipoid solvents. They seem to be distributed throughout the plastid in an exceedingly fine state of division. The distribution of chlorophyls through the stroma is easily disturbed. The absorption of water by the hydrophylic phase leads to the accumulation of the green pigments at the surface of the stroma. This is not a true separation of the two phases. A granular appearance of the chloroplast may be produced without a complete separation of the two phases. The appearance of the chloro-

plast is very much dependent upon the water relation of the hydrophyllic phase, and this should always be taken into account in cytological work. The chlorophyl may be separated completely from the plastid and crystallized, leaving a stroma which may have a porous foam-like structure.

V. *The Stability of Chlorophyl*

It is well known that alcoholic solutions of chlorophyl are quickly decomposed on exposure to light. In his work on the light relations of leaves, Wiesner thought that the amount of chlorophyl in the leaf remained constant under illumination, in spite of the rapid destruction indicated by alcoholic solutions, because there was a rapid resynthesis. This assumes that chlorophyl in the leaf is as rapidly destroyed as that in alcoholic solution. Reinke explained the constancy of the chlorophyl content of leaves by the combination of proteins with the green pigments to produce light stable compounds. Iwanowski seems to have proved that colloidal chlorophyl is far more light-stable than crystalline chlorophyl. Chlorophyl in colloidal solution in alcohol of low concentration is much more stable to light than chlorophyl in true solution in 80–90% ethyl alcohol. This seems a good indication that the light-stable pigments in the leaf are in colloidal condition.

VI. *Precursors of Chlorophyl*

Entirely insufficient information is available to make possible a prediction of the course followed in the chemical processes leading to chlorophyl formation. More is known about chlorophyl decomposition than about its synthesis. From the nature of the decomposition products we can gain only a meager outline of the steps in synthesis.

Most seedlings when grown in darkness do not form chlorophyl. If mature plant parts containing chlorophyl are put into darkness, the chlorophyl gradually disappears. The processes of chlorophyl decomposition may be enormously hastened by very low concentrations of ethylene, propylene, acetylene, and by some other gases. The concentrations of these gases required for chlorophyl decomposition indicates that they have a catalytic action only. Plant extracts containing chlorophyl are not decomposed by these gases even when they are bubbled through the extract in high concentrations. Wilting or bruising of the leaf is sufficient to stop chlorophyl decomposition by the unsaturated hydrocarbons. The temperature must be proper for active metabolism before the chlorophyl will decompose under exposure to these gases. The combination of ethylene with oxygen to ethylene oxide is sufficient to stop the blanching action on celery leaves. Propylene is a little more effective in chlorophyl decomposition than ethylene. Acetylene is active also, but it shows a greater toxicity to the cells than ethylene. The conditions of chlorophyl

decomposition are of commercial importance, and we must have more information on the mechanism of decomposition however complex it may be. Gardner has identified an enzyme which decomposes chlorophyl. The action of ethylene may be to hasten the action of this enzyme.

Many etiolated plants when grown in darkness do not possess chlorophyl, but they can form carotinoid pigments, anthocyanins, and flavones. However, chlorophyl is very quickly synthesized when the etiolated plants are exposed to light even of very low intensity. A considerable greening occurs in one or two hours' exposure of leaves to daylight. This indicates that well-elaborated precursors are formed in darkness, and that these are transformed to chlorophyl on exposure to light.

Monteverde and Lubimenko largely on the basis of absorption spectra suggest two steps in chlorophyl formation. The leucophyl of etiolated leaves is transformed into colorless chlorophyllogen and this under the action of light forms chlorophyl. The first step may proceed without the action of light. The last step generally requires light as a catalyst, but there are cases known which do not require light. The transformation of chlorophyllogen into protochlorophyl and other compounds indefinite in composition has been frequently suggested. It has at times been doubted that some of the green pigments found in certain plant parts are chlorophyl, because there seem to be differences in their properties. Even the green pigments of the stomata show some reactions different from ordinary leaf chlorophyl. That magnesium enters the chlorophyl under the catalytic action of light has been shown. This would indicate an introduction of the magnesium into organic combination late in the synthesis of the chlorophyl molecule, which is in keeping with the chlorophyl reactions with acids.

VII. *Chlorophyl in Chlorotic Leaves*

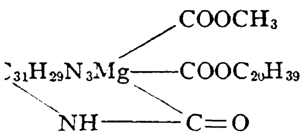
When there is a deficiency of iron in the nutrient medium, the leaves of plants grown therein will be light green in color, or chlorotic. Also, when magnesium or nitrates are deficient in the soil or nutrient solution, chlorosis will be observed. Iron is a catalyst in chlorophyl synthesis; magnesium and nitrogen are chlorophyl components; therefore all must be present for the formation of chlorophyl. The mosaic diseases are so named because they produce a mottled pattern of light-green and dark-green areas over the leaf surface. The disappearance of chlorophyl from the light-green areas seems to be brought about by parasites. In certain other diseased conditions, such as in the infection of flax with certain strains of *Fusarium lini*, the fungus may not appear above the cotyledons, yet these parts of the flax above the cotyledons may be entirely lacking in chlorophyl. The chlorophyl gradually disappears from the tips of the

leaves, then from the whole stem back as far as the cotyledons. Below that point the tissue remains green. Possibly the fungus produces some substance which diffuses upward and acts to destroy the chlorophyl, or the action of the fungus on the root may prevent the absorption of proper quantities of iron or magnesium. The former view is preferred because the chlorophyl may be first formed and then disappear.

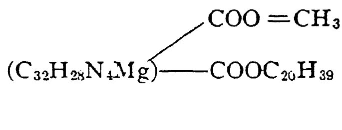
Very young leaves are somewhat deficient in chlorophyl on account of a deficiency of chloroplasts, but they become more green after leaf growth has ceased. The leaves of some ornamental plants show a chlorophyl deficiency which is inherited. Certain areas of leaves may be albino by inheritance.

VIII. *Chemical Reactions of Chlorophyl*

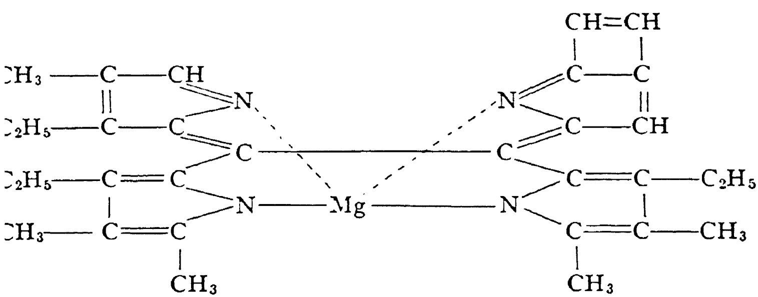
On reaction of chlorophyl with acids there is a quantitative removal of the magnesium without destruction of the nuclear group. With oxalic acid and other plant acids chlorophyl-a gives phæophytin-a as shown in the diagram, Fig. 61 (Table 21). To the magnesium in the chlorophyl has been ascribed a great importance in synthesis, since it is joined with the N of the pyrrol ring and forms a combination similar to its combination in the molecule of Grignard's reagent. Magnesium represents 5% of the chlorophyl. It is rather easy to substitute copper or zinc for the magnesium in phæophytin. The copper and zinc compounds of phæophytin are stable to light. The original colors of green plant parts can be preserved by treating the tissues with solutions of copper acetate.



chlorophyl-a



chlorophyl-b



ætiophyllin

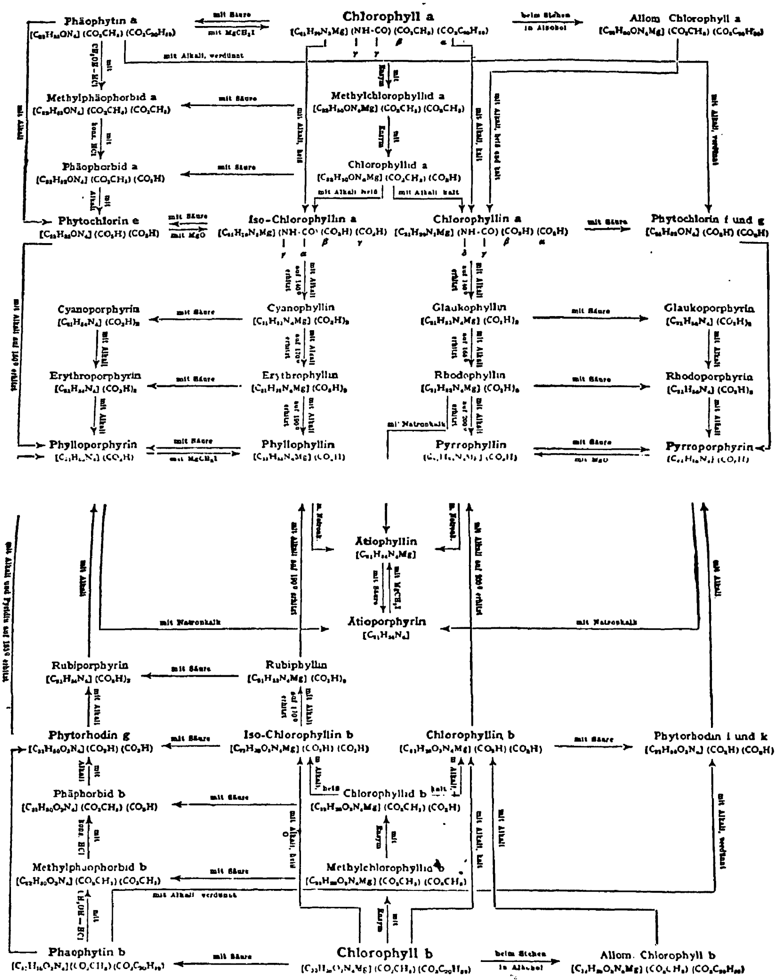


FIG. 61.—Scheme of the chemical reactions of chlorophyls. (After Willstätter.)

TABLE 21

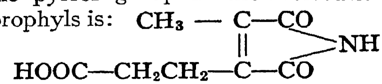
REACTIONS OF CHLOROPHYL

- Leaf Powder
  - ↓ (85% acetone)
  - ↓ Green sol'n of four pigments
    - → (ether + dist. H_2O)
      - ↓ Ether sol'n of pigments
        - → (methyl alc. potash)
          - ↓ Phase test brown to green—K salt of the acid chlorophyllin
        - → (30% K hydrate in methyl alc. + ether)
          - → Carotin and xanthophyl in upper ether layer
            - ↓ (petrol ether + 90% methyl alcohol)
              - → Xanthophyl in methyl alc.
              - → Carotin in petrol ether
          - → Saponified green pigments in lower layer
        - → (boiling 30% potash sol'n in methyl alc.)
          - ↓ Sol'n of K salts of isochlorophyllins
            - ↓ (H_2O — HCl + ether)
              - ↓ Ether sol'n of dissociated products
                - ↓ (extract 4% HCl)
                  - → Acid layer
                    - ↓ (NH_3 + ether)
                      - ↓ Phytochlorin-e From chlorophyl-a
                  - → Ether layer
                    - ↓ Extract 12% HCl + H_2O + ether
                      - ↓ Phytorhodin-g From chlorophyl-b
    - → (petrol ether + dist. H_2O)
      - ↓ Petrol ether sol'n of pigments
        - ↓ (90% methyl alcohol)
          - → Chlorophyl-a and carotin in petrol ether
            - ↓ (methyl alc. potash)
              - ↓ Phase test—yellow
                - → Carotin in ether layer
                - → Chlorophyl-a saponified in KOH layer
          - → Chlorophyl-b and xanthophyl in m. alc.
            - ↓ (ether + dist. H_2O)
              - ↓ Ether sol'n of chlorophyl-b
                - ↓ (methyl alc. potash)
                  - ↓ Phase test—brown red
                    - → Chlorophyl-b saponified in KOH layer
                      - ↓ (20% HCl + H_2O)
                        - ↓ Phæophytin (no Mg)
                          - ↓ (evaporate dissolve in alc. + Cu acetate)
                            - ↓ Cu compound of chlorophyl Cu substituted for Mg
                    - → Xanthophyl in ether

With alkalies the chlorophyls are converted to chlorophyllins which contain magnesium but from which the alcohols have been removed by saponification. Under the further action of alkalies the (NHCO) group is removed, then in order each COOH group is removed until finally ætiophyllin, $C_{31}H_{31}N_4Mg$, a compound without any carboxyls is produced from the chlorophyls. From this ætiophyllin the magnesium can be removed by acid to produce ætioporphyrin, $C_{31}H_{32}N_4$.

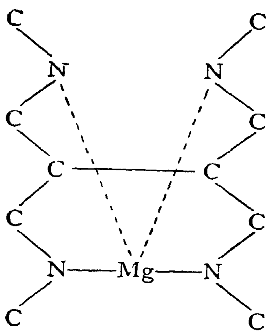
In testing to demonstrate the presence of two green pigments in the leaf extract, chlorophyl-a is transformed by treatment with acids to phæophytin-a, then with base to methylphæophorbid-a, and on further treatment with base to phæophorbid-a, then to phytochlorine-e which is olive green. Chlorophyl-b yields in the same manner phæophytin-b, methylphæophorbid-b, phæophorbid-b, then phytorhodin-g which is brick red. The differences in color produced by these reactions serve to identify the two chlorophyls.

The investigations of Marchelewski and of Willstätter (Fig. 62) point to the existence of the pyrrol group in the molecule. The chief oxidation product of chlorophyls is:

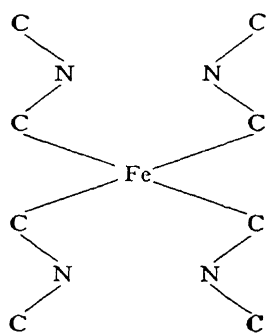


hematinic acid imide

Hematinic acid imide also has been obtained from hemoglobin of the blood. By the production of this substance the close chemical relationship between chlorophyl and hemoglobin seems to be established. The two compounds also yield a series of pyrrol derivatives which are the same. Possibly the relationship between the two compounds may be represented as follows:



chlorophyl nucleus



hemoglobin nucleus

Iron is required for chlorophyl formation and in the above formulæ for the nuclei of chlorophyl and hemoglobin magnesium replaces iron. Hence it seems that both chlorophyl and hematin may be derived from common precursors in whose formation iron is required. This might indicate that the iron-containing compounds originated first in nature in the evolution of the common ancestors of plants and animals. One is

FIG. 62.—Richard Willstätter, 1872–

"Without investigating chlorophyll itself, Willstätter and his collaborators deduced the characteristics of its constitution from a consideration of the derivatives that were formed upon reaction with acids and alkalies.

"If an alkali hydroxide is allowed to act upon chlorophyll it is converted into salts of a chlorophyll-green color. The neutral chlorophyll has become an acid which forms water-soluble salts. In the reaction with alkalies, therefore, there has been split off hydrolytically, without any significant optical change, a component which was bound with an acid group. The mild action of acid affects another part of the molecule so that the chlorophyll color is changed to an olive green; a salt-forming group is not formed, and therefore saponification is avoided. Hence, by acid cleavage one succeeds in sparing and in finding in the products of cleavage those components of chlorophyll that are split off by alkalies and conversely the alkali derivatives of the pigment must show another characteristic atomic group which is extremely easily destroyed by acids. As a consequence of this guiding conception it was possible, before chlorophyll itself was known, to combine its properties from the analysis of the decomposition products that are formed by acids and alkalies, and so perfectly was this done that when the preparation of the natural pigment in the pure state was finally successful nothing new was learned from the analysis of it."

Investigations on Chlorophyll. (By R. Willstätter and A. Stoll. Translated by F. M. Shertz and A. R. Merz.)

inclined to wonder if the iron bacteria had anything to do with the evolution of these compounds.

It is known that magnesium goes into the chlorophyl molecule late in the synthesis and that light is required for this introduction. The ease with which magnesium can be removed from chlorophyl by the organic acids, even by H_2CO_3, shows that its linkages do not hold together the complex chlorophyl molecule.

IX. *Carotinoid Pigments*

There are two classes of yellow pigments commonly found in leaf plastids. The carotins, $C_{40}H_{56}$, are hydrocarbons but evidently have very complex structure. The xanthophyls, $C_{40}H_{56}O_2$, are chemically closely related to them and possibly might be produced by oxidation from carotins. Yet the reactions of xanthophyl indicate that more probably all of the carotinoid pigments are built up from common precursors with a common nucleus. Xanthophyl shows no carbonyl, alcohol, or acid groups, and the oxygen seems to be in ether linkage.

There are at least two carotins of the same empirical formula. One, ordinary carotin, is yellow. It is commonly found in plants and is abundant in carrots. The other isomeric form called *lycopin* is yellowish red. It is commonly found in red tomatoes. There are at least three and possibly four xanthophyls which are isomeric forms of the same empirical formula. Tswett designated these as *xanthophyl* α, α^1, and α^{11} and *xantho-y*

These pigments all have widespread distribution in leaves, flowers, and fruits. They are commonly contained in special cell structures called *chromoplastids*. The carotinoids usually disappear upon disintegration of the plastids. The carotinoids may also be dissolved in oil droplets in the cytoplasm. In autumn leaves the carotinoids persist after the decomposition of the chlorophyl. In the coloration of some evergreen leaves evidently there is a production of lycopin, the yellow-red isomer of carotin. There may be present also in some conifers a red xanthophyl called *rhodoxanthine*. In brown algæ there is a pigment related to the xanthophyls and known as fucoxanthin ($C_{40}H_{54}O_6$). This also might be considered an oxidized carotin, but probably the relation is not so simple as that would imply, for the oxygen is not just in an additive compound.

The structural formula of carotin is evidently difficult to determine. Its empirical formula $(C_5H_7)_8$ is suggestive of a connection with terpene derivatives of the formula $(C_{10}H_{14})$ and possibly with isoprene (C_5H_8). The color of the hydrocarbon may be due to a chromatophore group $> C : C <$ in carotin.

The carotinoid pigments are very easily oxidized in air and take on oxygen to form addition compounds. Xanthophyl may add 42% of its original weight on oxidation to $C_{40}H_{56}O_{15}$.

The functions of the carotinoid pigments in the cell are somewhat in doubt. Evidently they are synthesized only in plants; animals are lacking in this ability. They seem to be merely casual constituents of animals derived from their food and not easily decomposed.

From a study of the presence of carotinoids and chlorophyl in albino

and virescent corn and rye seedlings, it seems that wherever chlorophyl is present in the seedling or wherever it may be formed in virescent seedlings, carotinoids also are found. But carotinoids may be present without chlorophyl formation being possible. This might seem to show that the carotinoids are related to precursors of chlorophyl, but that chlorophyl formation demands some agency in addition to the factors necessary for carotinoid formation. These factors are controlled by heredity evidently. The factor producing complete albinism in plants is lethal except in saprophytes such as *Monotropa*. Evidently part of the cells of a plant may be lacking in factors for chlorophyl formation and still function otherwise, if there is sufficient assimilating area elsewhere in the plant.

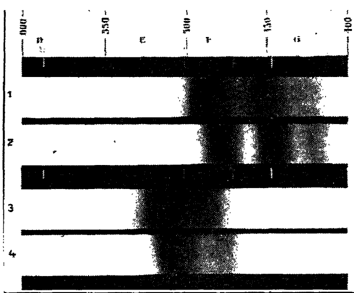


FIG. 63.—Absorption spectrum of yellow pigments. 1. Carotin in alcohol. 2. Xanthophyl in ... 4. Xan

Carotin and xanthophyl are generally quite constant in quantity in the leaf. There are usually about two molecules of xanthophyl for each molecule of carotin. The carotinoids have been stated to be reserves, but rather they are associated with stored reserves such as the lipoids in the spores of rusts. There is considerable doubt of the possibility of oxidizing these compounds as a source of energy. In the animal body they are usually excreted without oxidation. Kohl thought that carotin in carrot served as a reserve substance. Evidently if the presence of carotin should increase the spectral absorption of the leaf, the light so absorbed might be transformed to heat and raise the leaf temperature. However, the absorption bands of the carotinoids are in the blue region of the spectrum in which the energy content is not great. The absorption spectrum of carotin shows two distinct bands in the blue-violet region (Fig. 63). By the introduction of the oxygen of xanthophyl the position of these bands is shifted somewhat, but they have the same relative position. The yellow pigments may protect the deeper layers of the cell constituents from blue rays of the spectrum since they absorb strongly in this region. Iwanowski suggested that they protect chlorophyl from decomposition by blue light. Carotin protects diastase from destruction by violet rays of a wave-length of 420 $\mu\mu$.

Carotins and xanthophyls may function as oxygen carriers in the absorption of oxygen. Still they cannot be very vitally bound up with respiratory oxidations because colorless cells respire normally without carotinoids. The carotinoids may serve to regulate the oxidation poten-

tial in the cell through their ability to combine with large amounts of oxygen. They may function so in chloroplasts by carrying O_2 from chlorophyl-b to reduce it to chlorophyl-a. Willstätter and Stoll formerly proposed that carbon dioxide assimilation may be controlled by the equilibrium between chlorophyl-a and chlorophyl-b and that this equilibrium may in turn be controlled by the carotinoids. However, the relative constancy of the proportions of chlorophyl-a and -b would bring this in question. The system of reactions proposed assumed the oxidation of chlorophyl-a to chlorophyl-b and then reduction of the chlorophyl-b to chlorophyl-a by carotin which was oxidized simultaneously to xanthophyl. The oxygen of xanthophyl might then be liberated or reduced by other oxygen acceptors of the cell through the action of oxydoreductases. This assumes that the oxygen is carried through an elaborate series of reactions before it is liberated as molecular oxygen, which seems undesirable. Furthermore, there is the greatest doubt that xanthophyl is formed from carotin directly by oxidation. Probably carotins and xanthophyls do have the ability to reduce chlorophyl-b. There is a suggestion that fucoxanthine may perform the function of chlorophyl-b in the brown algæ which seem to be deficient in chlorophyl-b.

The fact that carotin can produce formaldehyde on exposure to light is no indication of its functioning so in photosynthesis, for many organic substances have this property. The fact that carotinoids are formed in etiolated plants indicates that they are not formed from chlorophyls. Yet all of these pigments according to Willstätter may have a common building stone isoprene, C_5H_8, which may form carotin or the phytol nuclei of chlorophyl.

It has been observed that the carotin content of carrots increased during the formation of starch from sugar. In the red tomato the accumulation of lycopin is generally coincident with the destruction of chlorophyl. Yet when the chlorophyl is removed by ethylene, lycopin is not always formed as a coincidence. The fruits may turn yellow on destruction of the chlorophyl and show the formation of red pigment only after several hours.

Tomatoes ripened at 30° C. or above develop little or no lycopin but may develop carotin and xanthophyl. The lycopin develops rapidly when the fruits are returned to a lower temperature. Oxygen is evidently essential to the development of lycopin. Possibly at high temperature, lycopin accumulation may be prevented by its oxidation.

CHAPTER XXIV

THE PHOTOSYNTHETIC REACTIONS

I. *Efficiency of Chlorophyl in Photosynthesis*

Willstätter and Stoll express the efficiency of the chlorophyl on the basis of the number of grams of carbon dioxide assimilated per hour divided by the chlorophyl in grams. They call the quotient the *assimilation number.*

$$\frac{CO_2 \text{ assimilated in grams per hour}}{\text{chlorophyl in grams}} = \text{assimilation number}$$

In normal leaves in summer the value of the assimilation number ranges from 5.8 to 9.1. In the spring in young leaves the value may rise to 14.2. In the autumn the efficiency is low. In leaves affected with mosaic disease the assimilation number is very low, evidently due to the effects of the parasite.

II. *Blackman Reaction*

In leaves which are rich in chlorophyl the sunlight intensity can be reduced to ½ or even to ¼ of the normal intensity with no slowing of the photosynthetic rate. This shows that the light intensity is in excess of the maximum that could be used in photosynthesis. When light and CO_2 are not limiting factors the photosynthetic process has a temperature coefficient $Q/10$ of 2 or 3 indicating that a chemical or enzymatic process limits the rate. This reaction is the *Blackman reaction.* The photocatalytic part of the process depends upon chlorophyl and light, while the enzymatic and condensation reactions depend upon the presence and activity of protoplasmic factors. In dark-green leaves the chlorophyl is in excess so that it is not a limiting factor. The action in strong light exposure is then limited by the rate of the reactions which may take place in darkness, that is by the Blackman reaction.

Photosynthesis does not consist in a simple photolysis of carbon dioxide, and photocatalytic reactions of carbon dioxide and water are not necessarily followed in photosynthesis. The primary process consists in the absorption of energy quanta by chlorophyl. This reaction has the temperature characteristic of a physical reaction. The rate of forma-

tion of the primary product (activated chlorophyl?) is proportional to the light quanta absorbed in unit time. The concentration of this primary product is determined by the algebraic sum of the rate of formation and the rate of its use.

The primary photosynthetic product may give up the stored energy quanta to an acceptor in secondary reactions. The acceptor is not CO_2 but probably a peroxide which is formed through an independent chemical reaction in the cell. In darkness this peroxide-forming reaction quickly reaches the equilibrium mixture of peroxide and the substances from which it was formed. The splitting of oxygen from the peroxide is the "dark" or "Blackman" reaction. Its temperature characteristic shows it to be not a physical photocatalytical process but a chemical reaction (Fig. 64).

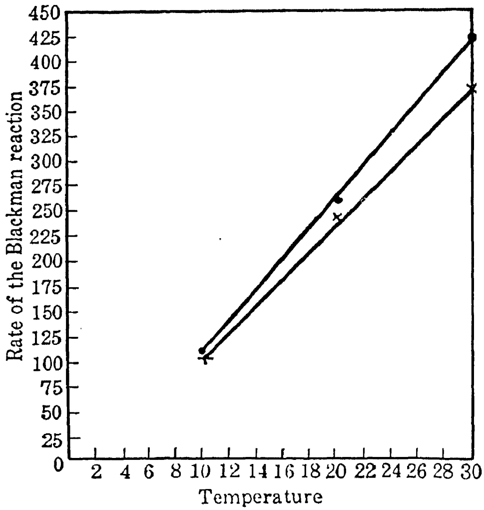


FIG. 64.—Relation of temperature to the rate of the Blackman reaction. (After Warburg.)

In light-green leaves the enzymatic process of condensing the photosynthate proceeds more rapidly than the photocatalytic process, so that the chlorophyl present may show a high efficiency per unit weight. A rise in temperature favors the action of the polymerizing enzyme and increases the photosynthetic rate when the enzyme activity is the factor that limits the photosynthetic rate, because the enzyme action has a high temperature coefficient. But when chlorophyl is deficient, temperature has little effect on the rate of assimilation, because this phase has a low temperature coefficient; under this condition a rise in light intensity will be effective in increasing photosynthesis.

Willstätter and Stoll considered that the condensing enzyme had its seat of action at the surface of the chlorophyl particle and that it was concerned with breaking down the intermediate peroxide-like compounds of CO_2 and chlorophyl with the release of oxygen and the production of the condensed product of photosynthesis. More than a single enzyme

may act. One may expect that one enzyme should have the ability of increasing the splitting of molecular oxygen from the chlorophyl-formaldehyde-peroxide compound much as catalase splits molecular oxygen from hydrogen peroxide. There may be present more than a single enzyme for the polymerization of the formaldehyde.

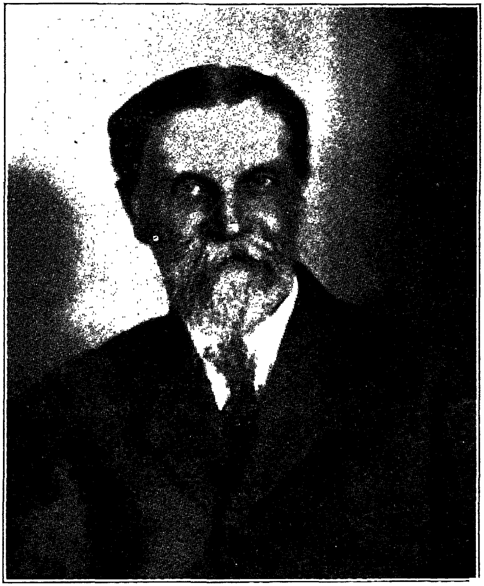


FIG. 65.—Clemente Arkadevitch Timiriazev, 1842–1920.

leaf exposed during a suitable time to the light of a spectrum of sufficient intensity would show a deposit of starch strictly in accordance with the absorption spectrum of chlorophyll."

Enregistrement photographique de la fonction chlorophyllienne par la plante vivante. Comptes rendus, Acad. Sci. Paris. 110: 1346–1347. 1890.

III. *Light Absorption by the Green Leaf*

Timiriazev (Fig. 65) showed that there is a close agreement between the light absorbed by chlorophyl and the wave-lengths effective in photosynthesis. The transmission spectrum of the green leaf and of its alcoholic extract show essentially the same characteristics. There is complete absorption of wave-lengths greater than about 7,000 Å. with an abrupt shading into a strong transmission band beginning at about the B Fraunhofer line of the spectrum. This band continues to about 6,750 Å., when

there is abrupt shading into a strong absorption band. The strong transmission band in the red is striking. It is the last part of the spectrum to disappear as the quantity of chlorophyl before the spectroscope is in-

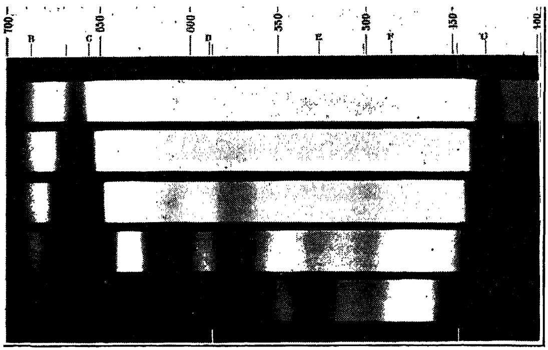


FIG. 66.—Absorption spectrum of chlorophyl-a. (After Willstätter.)

creased. Very concentrated layers of the green pigments in solution show transmission of this band and appear red. There is a maximum absorption of the visible spectrum at about the C line. Beyond this there are four minor absorption bands, the first in the orange, the second in the greenish

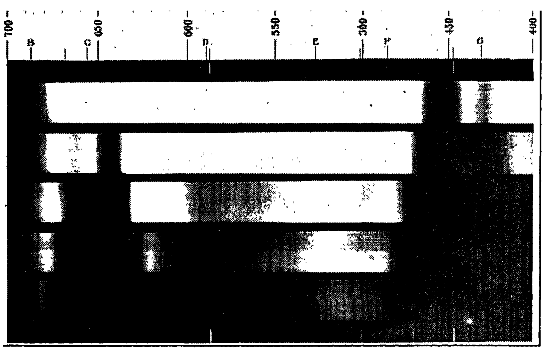


FIG. 67.—Absorption spectrum of chlorophyl-b. (After Willstätter.)

yellow, and two fainter bands in the blue and indigo. Practically complete absorption occurs at wave-lengths shorter than the G line.

The absorption spectrum of the leaf is the resultant of the absorption of all of the leaf pigments. The portions absorbed by each component

can be seen from the absorption graphs of chlorophyl-a (Fig. 66) and chlorophyl-b (Fig. 67).

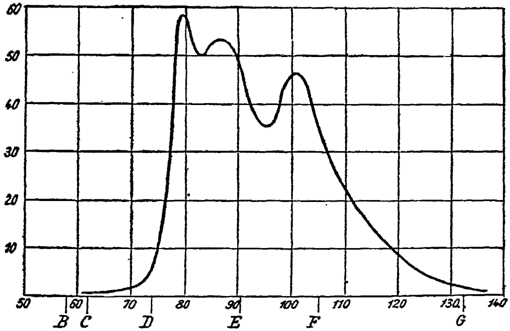


FIG. 68.—Absorption curve of a phycoerythrin solution.

Chlorophyl solutions show in thin layers a green color by transmitted light, but a red fluorescence by reflected light. Chlorophyl has the ability

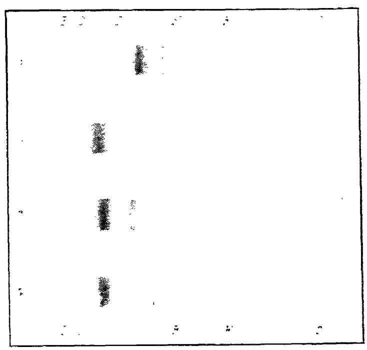


FIG. 69.—Absorption spectra. 1. Phycoerythrin. 2. Blue-green phycocyanin. 3. Blue phycocyanin. 4. Blue-violet phycocyanin.

to absorb light of short wave-lengths and reradiate light of a longer wave-length. The principal wave-lengths reradiated from the green cells of leaves extend from about 7,000 Å. to 6,750 Å. This region lies in the region of high transmission by the green leaf. The fluorescence spectrum of chlorophyl dissolved in alcohol has a maximum at 6,540 Å. When it is dissolved in lecithin, the fluorescence maximum lies at 6,770 Å. This very closely approaches the maximum fluoresence shown by most leaves and indicates that the chlorophyl in the leaf may be present in solution in lecithin or some closely related substance.

IV. *Absorption Spectra of Green, Blue-Green, Brown, and Red Algæ*

In addition to chlorophyl and the carotinoids there are commonly present in some algæ, pigments which modify their absorption spectra. The red algæ contain a red pigment called *phycoerythrin* (Figs. 68, 69).

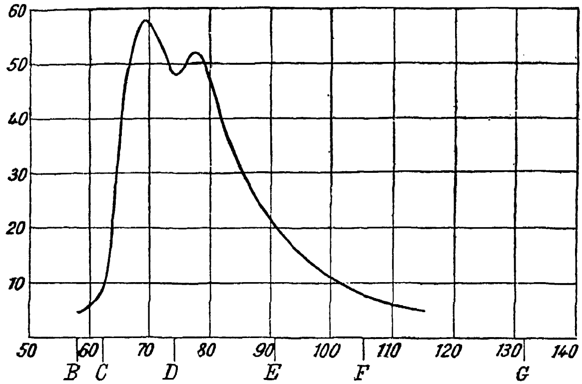


FIG. 70.—Absorption curve of blue phycocyanin.

The CYANOPHYCEÆ contain the blue-green pigments called *phycocyanins* (Figs. 69, 70, 71). The phycocyanins are the characteristic pigments of

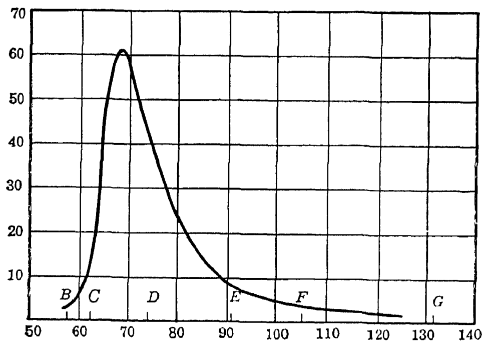


FIG. 71.—Absorption curve of blue-green phycocyanin.

the CYANOPHYCEÆ, but they occur also in the RHODOPHYCEÆ. Evidently there are at least three phycocyanins which differ in their absorption spectra. A blue-green phycocyanin occurs in *Calothrix* and other species of CYANOPHYCEÆ, and also in *Batrachospermum* of the RHODO-

PHYCEÆ. Crystals and solutions of this pigment are blue to bluish-green, and solutions show a carmine-red fluorescence. A bright-blue phycocyanin occurs widespread in the CYANOPHYCEÆ. Good preparations can be prepared from *Phormidium sp.* There is a blue-violet phycocyanin which can be prepared as dichroic rhombic crystals from *Ceramium rubrum* and *Porphyra hiemalis*. Variations in the proportions of these pigments change the absorption spectra of the algæ.

TABLE 22

TRANSMISSION COEFFICIENTS OF LIGHT OF DIFFERENT WAVE-LENGTHS FOR PURE WATER

Per cent transmitted to depths meters noted

Wave-length	1M	5M	10M	20M	25M	30M	35M	50M	75M	100M
300.0	85.81	46.5	21.8	4.6	2.1	1.0	.47	.047	.001	.00300
415.0	96.59	84.0	70.6	49.9	42.0	35.2	29.0	17.6	7.6	3.1
420.0	97.74									
449.0	98.81	94.2	88.7	78.7	74.1	69.9	65.8	54.9	40.7	30.3
450.0	98.03									
468.0	98.81	94.2	88.7	78.7	74.1	69.9	65.8	54.9	40.7	30.3
470.0	96.67									
475.0	98.03	90.5	81.9	67.1	60.8	55.0	49.8	37.1	22.5	13.6
494.0	97.06									
506.0	98.03	90.5	81.9	67.1	60.8	55.0	49.8	37.1	22.5	13.6
510.0	97.54									
522.0	98.52	97.8	86.1	74.2	68.8	63.9	59.3	47.4	32.6	22.5
525.0	97.06									
537.0	99.50	97.5	95.1	90.5	88.2	86.0	83.9	77.8	68.6	60.5
539.0	97.93									
550.0	96.48	83.6	69.9	48.8	40.8	34.1	28.5	16.6	6.85	2.7
558.0	96.48									
562.0	97.06	86.1	74.2	55.1	47.4	40.8	35.3	22.5	10.68	5.06
575.0	98.03									
579.0	94.59	75.7	57.3	32.8	24.8	18.8	14.3	6.1	1.54	.38
587.0	95.06									
589.5	90.90	62.0	38.5	14.8	9.19	5.7	3.5	0.85	0.075	.0071
600.0	85.04									
600.5	84.87	44.0	19.3	3.76	1.6	.72	.32	.027	.0045	
610.2	82.68									
618.0	81.48	35.9	12.9	1.6	.59	.21	.077	.0035		
625.0	80.10	32.9	10.8	1.18	.39	.12	.0429	.0015		
630.0	79.96	32.6	10.6	1.14	.38	.12	.0396	.0013		
637.0	79.65									
640.0	77.61	28.0	7.92	.627	.18	.049	.014	.00013		
650.0	78.68									
662.0	75.64	24.7	6.11	.37	.09	.023	.0056	8.06×10^{-8}		
663.0	78.40									
675.0	72.10	19.48	3.79	.141	.028					
687.0	70.35	17.23	2.96	.088	.0151	.00045	2.31×10^{-6}			
700.0	57.83	6.4	.40	.00167	.00107	6.8×10^{-6}				
779.0	1.70×10^{-10}									

These pigments, phycocyanin and phycoerythrin, seem to be protein in nature. The pigment evidently contains an albumin joined to the pigment group in somewhat the same manner as hemoglobin. The

presence of phycoerythrin may overbalance the color of other pigments in the red algæ so that the absorption maximum lies between the D and E lines of the spectrum and the absorption between the B and C lines is very weak in comparison with the absorption spectra of green algæ.

In the CYANOPHYCEÆ the absorption maximum lies at the D line in the orange. In the brown algæ there are two regions of high absorption; between the D and E lines the absorption is a little greater than between the B and C lines.

The red, green, brown, and blue-green algæ show a difference in their distribution in the ocean. The green algæ are mostly surface or shore forms. The blue-green algæ are somewhat variable and seem adaptable to different depths. The brown algæ are littoral forms, while the red algæ penetrate to the greatest depths into the sea.

This distribution is at least somewhat in relation to the rays of light which can penetrate to the depths at which the algæ grow (Table 22). Water quickly absorbs the heat rays and longer wave-lengths of the red part of the spectrum. The short wave-lengths penetrate to the greatest depths into water. The red algæ growing at great depths are of a color such that they can absorb the light which reaches them. The red phycoerythrin absorbs the blue and violet wave-lengths. This relation between the light penetration into sea water and the ability of algæ having colors complementary to this light led Engelmann to formulate his hypothesis of complementary chromatic adaptation. According to this idea the red algæ are able to survive at great depths in the sea because they possess phycoerythrin which absorbs the light which penetrates down to them. The green algæ are excluded from this region by their inability to absorb short wave-lengths of light so efficiently as the red algæ.

The blue-green algæ are adaptable to different light qualities, and they are the best examples of complementary chromatic adaptation. When *Oscillatoria* is exposed to light of one color it tends to assume a color complementary to the color of the light, so that the light is more efficiently absorbed. In red light *Oscillatoria* is green in color, in green light it turns red, in yellow light it becomes blue-green, and in blue light brownish yellow. This complementary adaptation to the color of the incident light is brought about by the appearance within the cells of different proportions of the two pigments, phycoerythrin and phycocyanin. These pigments evidently fluctuate within rather wide proportions in some blue-green algæ. The ability to adapt themselves to different wave-lengths of light would be of advantage not only in the ocean, but also under the forest cover where the light which penetrates shows a higher content of green wave-lengths, and under the dense cover of

pond weeds through which scarcely any light penetrates but the dark red band transmitted by chlorophyl.

V. *Radiant Energy and Photosynthesis*

There is evidently an adaptation of plants not only to the quality but also to the intensity of light. Quite commonly we know that some plants are shade-loving (*heliophobous*) or will grow only in strong insolation (*heliophilous*). Such plants as maidenhair fern, fir, yew, beech, and linden are more adapted to living in shade than in strong insolation. The jack-pine, larch, birch, locust, and plants of the prairie are adapted to strong insolation. Shade plants generally have a high content of chlorophyl. Their chloroplasts give evidence of the injurious effects of too strong insolation. In many plants from the algæ to the dicotyledons there are special adaptations for decreasing the light which falls upon the chloroplasts. In *Mougeotia* the chloroplast is a flat band lying in the center of the cell. In low light intensity the chloroplast lies at right angles to the incident light. When the light is increased excessively, it turns parallel to the incident rays. The chloroplasts of many higher plants are lens-shaped. In low intensities of light they occupy mostly the top and bottom of the palisade cells with the greater diameter exposed to light. But they may be moved with the cytoplasm to the lateral walls in intense insolation. In this position only the edge of the chloroplast is exposed to the light (Fig. 72). There are many good evidences that the noonday sunlight is too intense for the optimum rate of photosynthesis.

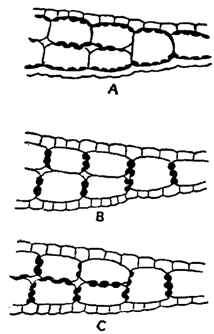


FIG. 72.—Transverse sections through the leaf of *Lemna trisulca* showing the position assumed by the chloroplasts in (A) light of moderate intensity, (B) intense light, and (C) darkness. (After Stahl.)

At the upper limit of the atmosphere there arrives light energy equivalent to 1.94 calories per sq. cm. per minute. The highest value recorded from 1918 to 1925 was 1.965 and the lowest value 1.909. The value fluctuates with changes in the sun's radiation. The noonday intensity at the earth's surface is much less than this value since the atmosphere absorbs and reflects about 65% of the incident energy. The maximum solar radiation varies through the year as shown in Fig. 73. The light intensity corresponds to about 9,250 foot candles at noon on June 21 at latitude 42° N.

The radiant energy which plants receive shows its greatest heat value in the region of the spectrum below the visible rays. The visible rays represent only a small fraction of the energy received. The actinic rays which are effective in producing chemical transformations represent an

exceedingly small part, owing to their lesser abundance in the sun's light and also to high refraction and reflection in the atmosphere. The ultra-violet rays are mostly lacking except in the summer and when the

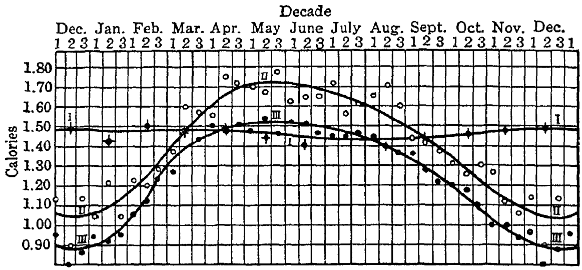


FIG. 73.—Maximum solar radiation per minute in gram calories per square centimeter at Washington, D. C. I. Solar radiation at normal incidence. II. Solar and sky radiation on a horizontal surface, with clouds near the sun, but not obscuring it. III. Solar radiation on a horizontal surface, with cloudless sky. (From Kimball.)

sun is at a high angle. Fig. 74 gives the relation of the sun's height to the quality of light transmitted through the atmosphere.

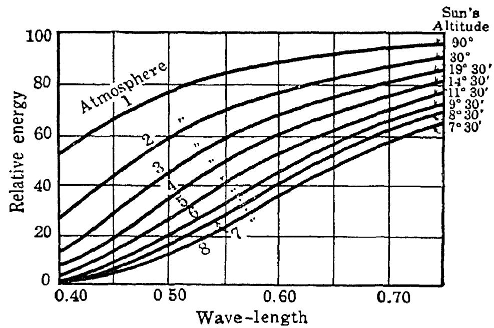


FIG. 74.—Showing the variation in the spectral character of sunlight due to atmospheric absorption.

The energy distribution in the light from an electric lamp is shown in Fig. 75.

VI. *Energy Storage in Photosynthesis*

The energy stored in the photosynthetic process was measured by Sachs by determining the increase in dry weight of leaves exposed to light and then calculating the energy storage from these data. The rate of the photosynthetic process can be measured also by finding the amount of CO_2 utilized or of O_2 produced.

Probably one of the best methods is to determine the heat of combustion of the substances produced by the leaves. This method avoids the difficulty of making a determination of the quantities of various substances produced, whether carbohydrates, fats, or proteins. The heat of combustion of compounds is expressed in calories per gram or per gram-molecule liberated when the compound is completely oxidized by oxygen. The unit of heat energy, the calorie, is taken as the energy required to increase the temperature of one gram of water from 14.5° to 15.5° C.

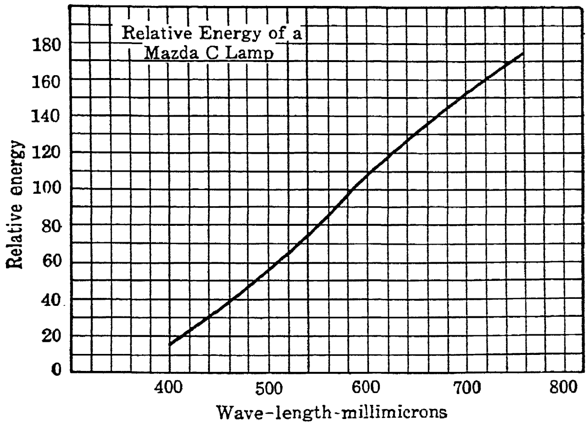


FIG. 75.—Relative energy output of a clear Mazda C lamp.

In the process of formation of the photosynthate, light energy is stored; the process is endothermic. It is a reaction involving the reduction of CO_2. The heat of the reaction is negative, that is, energy must be added to cause the reaction to proceed. We wish to find what percentage of the light which falls on the leaf is converted to chemical energy in the reaction.

The great numbers of chemical substances formed by plants differ markedly in their energy content. In general, the plant constituents can be divided upon the basis of the chemical nature and heat energy value into carbohydrates, proteins, and fats. The relative energy values of these substances may be illustrated by the following examples:

TABLE 23

Substance	*Calories per gram*
Glucose	3.74
Sucrose	3.95
Starch	4.18
Cellulose	4.18

TABLE 23—*Continued*

Substance	*Calories per gram*
Legumin	5.62
Globulin	5.60
Hordein	5.92
Olive-oil	9.45
Linseed-oil	9.43

The most condensed storage form is fat. It has the greatest heat value per gram. More energy is required to form fat, and more heat is evolved when it is burned by respiration or ordinary combustion, than is evolved from carbohydrate or protein. From the relationship between the sugars and starch it is evident that the energy per gram is increased when the anhydride is formed, yet the increase is not great.

The molecular heats of combustion of three typical plant substances are as follows:

Glucose	$C_6H_{12}O_6$	per mol	677×10^3 cal.
Leucine	$C_6H_{13}O_2N$	per mol	857×10^3 cal.
Stearic acid	$C_{18}H_{36}O_2$	1/3 mol (C_6)	903×10^3 cal.

If we use the increase of dry weight as a measure of the rate of photosynthesis, we must take into account the nature of the substance formed.

In the process of photosynthesis the first step is the solution of the CO_2 of the air in H_2O of the leaf to form H_2CO_3. In this reaction very little energy is involved, and hence the reaction is easily reversible. The carbonate ions of the leaf are maintained at a fairly constant concentration by equilibrium with the CO_2 of the atmosphere. If one considers that formaldehyde is the first product of photosynthesis and the polymerization of this to a hexose then follows, the principal energy storage reaction of photosynthesis is this formation of formaldehyde. Six molecules of formaldehyde yield on combustion 733.2×10^3 calories. The heat of combustion of the equivalent molecule of glucose yields 677.2×10^3 calories. Hence if formaldehyde is polymerized to glucose, there is a liberation of 56×10^3 calories for each molecule of glucose formed. The polymerization reaction is exothermic.

The energy of the light which falls on the leaf may be determined by various means. The bolometer is excellent for determining the energy distribution in different parts of the spectrum. The silver disk pyrheliometer is a convenient instrument for use in the field where the total radiant energy is to be determined. The Macbeth illuminometer is a convenient and accurate instrument for the measurement of light in-

tensities. The photoelectric cell and recording potentiometer is an excellent means of obtaining continuous records of light fluctuations.

Various attempts have been made to determine the efficiency of the leaf in energy storage by photosynthesis. Most of these estimates are very unfair to the efficiency of the leaf, for they do not give proper allowance for the fraction of the energy absorbed by the leaf. The estimates of the efficiency of the leaf as an organ for the storage of energy vary from .6 to 60% of the incident light energy being transformed into chemical energy. In the alga *Chlorella* the efficiency was estimated to be as high as 61%. Many of the estimates do not take into account the fact that the light incident upon the leaf may have been in excess and therefore not limiting, or even injurious to the photosynthetic action.

By using continuous artificial light which was controlled at an intensity which averaged for the 24-hour period about 292 foot-candles, Eaton found that in tobacco plants about 6.5% of the light energy received in the visible part of the spectrum was stored in combustible compounds. The energy values were determined from the heat of combustion method. The temperature (70° F.) and humidity were also controlled in this experiment. By measuring the volume of water transpired it was found that 45% of the energy was used in the evaporation of water.

Probably the best estimates of the efficiency of use of energy in photosynthesis is that made by Warburg and Negelein on the alga *Chlorella*. The conditions for photosynthesis were considerably better controlled than can be done in the case of air leaves, exposing the alga in water in a cell with silvered sides which was supposed to reflect back all the rays diffracted or scattered. A very dense suspension of the *Chlorella* was used so as to absorb all of the incident light. By the use of screens, light of the following wave-lengths was separated: red, 690–610 $\mu\mu$; yellow, 578 $\mu\mu$; green, 546 $\mu\mu$; blue, 436 $\mu\mu$. The red and yellow light were about 97% absorbed, the green was about 90% absorbed, and the blue was about 99% absorbed by the cells. The intensity of the incident light was measured by the bolometer. The rate of photosynthesis was measured by calculating the energy utilized from a determination of the oxygen evolved. If v c. c. of oxygen are evolved, the energy utilized is assumed to be $v\,\frac{112{,}300}{22{,}400}$ calories. This is the value to be assumed if the whole of the CO_2 is completely transformed into hexose with the requisite amount of water and the evolution of oxygen.

It was found that the efficiency of the use of the energy in photosynthesis increased as the light intensity decreased. Consequently, the efficiency was measured at the lowest possible light intensities and at higher intensities. Then by plotting a graph of the efficiency at these

various intensities a curve was given which on exterpolation gave the efficiency if the light intensity were taken at zero. For the different wave-lengths used the calculated efficiency in percentage of the total when the light intensity was at zero is given in the following table:

Wave-lengths in $\mu\mu$	
660	59%
678	53.5%
546	44.4%
436	33.8%

The efficiency of different wave-lengths of light decreases with the decrease in wave-length. The red rays are most efficient in photosynthesis. By fluorescence of the chlorophyl the yellow, green, and blue wave-lengths are transformed into red wave-lengths from 700 $\mu\mu$ to 675 $\mu\mu$ and reradiated. This band in the red has a relatively higher efficiency than the original wave-length. There is a transformation of the wave-lengths from the original, so that we may not have to do with the action of the original wave-length but with the efficiency of red light produced by fluorescence.

Adams has recalculated the data of Warburg and Negelein on the basis of the following reaction: $CO_2 + 3H_2O \rightarrow 2H_2O_2 + HCHO$. The heats of combustion of all of the substances are taken as in solution in water as follows:

Heat of combustion of one molecule gaseous formaldehyde, +137.0 Calories.

Heat solution of one molecule of gaseous formaldehyde, +15.0 Calories.

Heat of solution of CO_2, one molecule, +5.6 Calories.

Heat of formation of H_2O_2, one molecule (in solution), 21.7 Calories $-137.0+15.0-5.6-(2\times 21.7)=-171.0$. The number of Calories per molecule required in the reaction are therefore: 171.0.

Six molecules of formaldehyde are required for the formation of one molecule of glucose. The heat of combustion of glucose is 677.2 Calories. The heat of solution is +3.2 Calories. Then one molecule of glucose in solution represents 674 Calories.

The maximum absorption in the red shown by chlorophyl-a is at λ666 mμ and by chlorophyl-b, λ640 mμ. Then if the value for $\frac{cNh}{J} = 28.46$ Calories, the quantum values at the maximum absorption values of the two chlorophyls are

Chlor. a, λ666 mμ = 42.7 Cal. per molecule
Chlor. b, λ640 mμ = 44.5 Cal. per molecule

Warburg and Negelein found slightly more than four quanta of red

light absorbed by Chlorella per molecule of CO_2 assimilated. If it is assumed that the absorption by both chlorophyls is equal and that each absorbs two light quanta, then the energy absorbed should be $2 \times (42.7 + 44.5) = 174.4$ Calories per molecule. This is slightly greater than that required for the above reaction. The maximum efficiency on the basis of glucose would be $\frac{674}{(6 \times 174.4)} = 64.4\%$. Warburg and Negelein's results become for the average efficiency in the red (59%) $59 \div 64.4 = 91.6\%$.

The formula for chlorophyl-a assumed by Willstätter is
Instead of Willstätter's formula for chlorophyl-b,
g, Adams proposes $C_{56}H_{72}O_6N_4Mg$.

Experiment has shown that in crude chlorophyl solutions protected from carbon dioxide and exposed to light, the chlorophyl-b absorption tends to disappear, and reappears when the solution is exposed to air and light. Willstätter and Stoll found the ratio, chlorophyl-a, chlorophyl-b, consistently greater for leaves growing in sunlight than for leaves growing in the shade. They give for the mean values of the ratio, respectively, 2.93 and 2.61, or a difference of 0.32, but if the results for the different species be weighted according to the number of observations, instead of being given equal weight, the mean values of the ratio become 2.93 and 2.43, respectively, and the difference, 0.50. Their data also show that on cloudy days the ratio diminishes, that is, tends toward the "shade" value. It is to be expected that during active photosynthesis the local carbon dioxide concentration would be lower than in the general atmosphere.

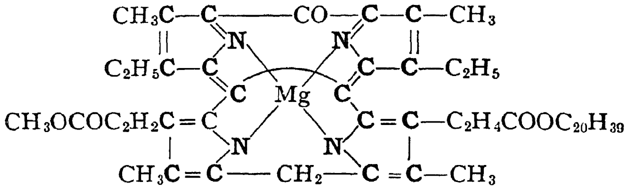
The chlorophyl may be pictured as going through a cycle of four reactions, two of them associated with the absorption of two quanta each of radiation, and two follow-reactions requiring water and carbon dioxide, but not light:

( ) Represénts ($C_{55}H_{72}O_4N_4Mg$)
∴ ( ) O is Chlorophyl-a ( ) CO_2 is Chlorophyl-b

$$( )\,O + H_2O + 2\text{Quanta } \lambda 666\ m\mu \rightarrow ( ) + H_2O_2$$
$$( ) + CO_2 \rightarrow ( )\,CO_2$$
$$( )\,CO_2 + H_2O + 2\text{Quanta } \lambda 640\ m\mu \rightarrow ( )\,CO + H_2O_2$$
$$( )\,CO + H_2O \rightarrow ( )\,O + HCHO$$

As to the 2-quantum absorption, two types of explanation are available: first, the teleological one, since the energy required for either stage of the photosynthetic process exceeds the quantum for the wave-length of light available to plants, the plants were obliged to develop substances which could pick up two quanta at a time; second, from the structure of chlorophyl.

The structure given by Adams is slightly different from that given by Willstätter for chlorophyl-a, but the difference does not affect the discussion to follow. The two conjugate chains of eleven atoms each may absorb a quantum of red light. The differences between chlorophyl-a and chlorophyl-b do not involve these two chains, so only a slight difference in wave-length of light absorbed is to be expected.



Chlorophyl-a

The light energy falling upon the leaf may be disposed of by photosynthesis, transpiration, transmission or reflection, and thermal emission to the surroundings (Fig. 76). Brown and Escombe estimated the energy budget of the leaf of *Polygonun weyrichii* under various conditions to be as follows:

0.42– 1.66% photosynthesis
9.67–57.01% transpiration
35.28–35.31% transmitted
6.01–54. 6% emitted

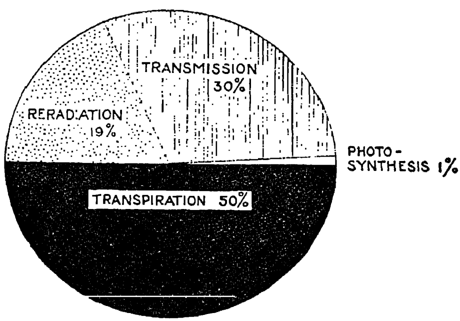


FIG. 76.—What happens to solar radiation incident on a chlorophyllous leaf. The values indicated give approximate disposal of the energy; the ratio varies with changes in external conditions. (After Spoehr.)

Puriewitsch estimated the efficiency of foliage leaves under various conditions to lie between 0.6 and 7.7%.

The percentage of the energy dissipated by transpiration was estimated by Briggs and Shantz as follows:

Wheat	54%
Barley	73%
Millet	85%
Alfalfa	80%

The energy so dissipated can be very closely approximated by weighing the water evaporated. If the energy used in transpiration is added to that used in photosynthesis the efficiency of the plant is rather high.

Upon the basis of material economy the plant is more efficient than most syntheses of organic chemistry. The leaf evidently gets a 100% yield from the CO_2 absorbed.

PRODUCTS OF PHOTOSYNTHESIS

I. *First Product of Photosynthesis*

Ever since the synthesis of a sugar by Butlerow from formaldehyde and the advancement of Baeyer's hypothesis of the photosynthetic reaction, it has been a favorite idea among chemists that a hexose was the first sugar formed in carbon assimilation. Like Baeyer's hypothesis this idea seems to have gained credence on account of its chemical simplicity and because it can be demonstrated in the test-tube. But this is no demonstration that a hexose is formed in the plant. In fact, the most careful analyses seem to indicate that not a hexose but sucrose is the first sugar appearing in the leaf. Brown and Morris concluded that sucrose is the first sugar of the leaf and that this sugar functions as a temporary reserve. When the sucrose of *Tropæolum* leaves reaches a certain threshold value, starch formation begins. This involves hydrolysis and enolization of the fructose fraction to glucose before polymerization to starch. Although Sachs (Fig. 77) demonstrated starch as the first visible product of photosynthesis and proved that its formation depended upon the conditions requisite for photosynthesis, it does not appear in photosynthesizing cells for about five minutes after exposure to light, whereas oxygen evolution begins at once. It is hardly to be expected that so insoluble and unreactive a complex substance as starch would be the first product of photosynthesis. That a substance of the general formula $(CHOH)_N$ is formed is practically proved by the photosynthetic ratio $\frac{CO_2}{O_2} = 1$.

$$nCO_2 + nH_2O \rightarrow (CHOH)_n + nO_2$$

n molecular volumes	n molecular volumes

If other organic substances such as oils, acids, or other substances with empirical formulæ different from $(CHOH)_n$ were formed, the ratio would not be unity.

Parkin working on the snowdrop and Davis, Daish, and Sawyer working on the mangold leaf agreed with Brown and Morris that sucrose

is the first sugar produced. To support this view they state that sucrose is always present in relatively high proportion in the leaf. The sucrose is inverted by sucrase, which is secreted by or is distributed around the sieve-tubes. As one passes farther away from the photosynthetic region

FIG. 77.—Julius von Sachs, 1832–1897.

"I concluded . . . that the starch in the chloroplast is not only a secondary deposit, but that it must be considered as the product of the assimilation ability of the chlorophyll through the agency of light, that it is formed here from its separate constituents, and is conducted from here out to the growing bud parts and to the tissues which accumulate reserve materials.

"If the seedlings developed in darkness, after the complete exhaustion of the starch, are placed in the light, the yellow chloroplasts in the first place turn green; if the light is intensive enough and acts sufficiently long, then starch grains form in the green chloroplasts, if the action of the light is not intensive enough, the chloroplasts turn green without the formation of starch in their interior.

"If no starch appears in the green chloroplasts on account of too low light intensity, then the plants die as if in darkness; if on the other hand the light intensity is sufficient to produce starch in the chlorophyll, then this is distributed into other parts, principally into the buds, and these now begin further to grow.

"From these facts it follows that the growth of the bud parts is dependent upon the formation of starch in the chlorophyll of the leaves. From the conditions, that the first formation of starch begins in the chlorophyll and that only the plant parts containing chlorophyll have the ability to evolve oxygen, it follows that the starch formed in the chlorophyll is formed here through assimilation, that is, it is formed out of inorganic substances; and that, on the other hand, no starch forms in the other non-green plant parts, but that it is transported into them, while the assimilation of the organic materials necessary, therefore, takes place in the chlorophyll-bearing cells of the leaves."

Ueber den Einfluss des Lichtes auf die Bildung des Amylums in den Chlorophyllkörnern. Botanische Zeitung, 20: 365. 1862.

of the plant the hexoses increase in proportion, owing to the inversion of sucrose for translocation.

During the day (Figs. 78, 79) the content of hexose in the leaves remains fairly constant, but sucrose fluctuates greatly. In the mangold leaf the hexoses fluctuate but do not show the regular periodic trend shown by sucrose. Sucrose increases during the day and diminishes during the night. It seems to be a temporary storage product. The possibility of maltose being the first sugar of photosynthesis is excluded by the meager quantities found in the leaf. Even when maltose is formed

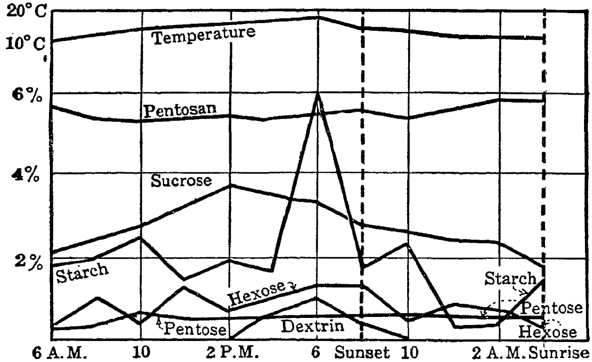


FIG. 78.—Variation in content of various carbohydrates in the leaf of potato during 24 hours, July 16–17, 1914. (After Davis, and Sawyer.)

from starch, maltase seems to be so greatly in excess that maltose is not ordinarily abundant in the leaf. It increases in quantity during starch digestion.

Probably the best argument for sucrose being the first sugar of the leaf is that based on the work of Krasheninnikov. A definite relation seems to hold between the amount of carbon dioxide decomposed and the dry weight laid down by the leaf. The increase in dry weight for each unit weight of carbon dioxide decomposed is as follows:

Bamboo	0.60
Cherry laurel	0.60
Sugar-cane	0.67
Linden	0.74
Tobacco	0.68

The formation of a carbohydrate of the composition $C_{12}H_{22}O_{11}$, like sucrose, would give a value of 0.64 in good agreement with these data. The value if glucose were the first product should be a higher dry weight per unit of CO_2 decomposed.

In the early stages of growth sucrose is the principal substance present in the leaf, whereas glucose would be expected to be more prominent if it were the first product of photosynthesis. De Vries by microchemical tests came to the conclusion that sucrose was the first sugar of the leaf.

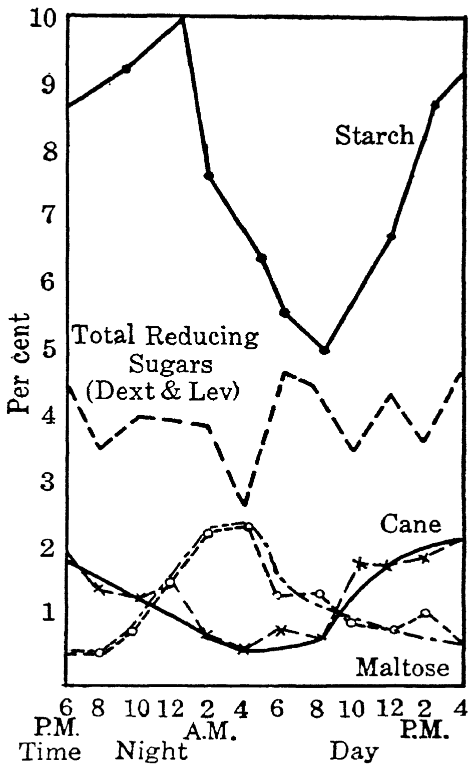


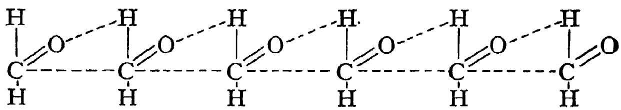
FIG. 79.—Carbohydrates in mangold leaf: percentages at two-hour intervals.

The best argument for the first product of photosynthesis being a hexose is its greater simplicity, but even here difficulty is encountered, for we should have to say which hexose appears first, or whether glucose and fructose are formed simultaneously or one is formed from the other by enolization to produce the secondary product, sucrose. This difficulty does not arise if sucrose is taken as the first product. Information on

whether the condensation habit of formaldehyde is into three, six, or twelve unit aggregates would help greatly in the solution of these questions.

II. *Synthesis of Sugars in Photosynthesis*

Following the synthesis by Butlerow of a sugar from formaldehyde, Baeyer (1870) proposed that formaldehyde was produced by plants and condensed to sugars in a manner similar to synthesis which can be produced in the test-tube. Six molecules of formaldehyde could condense to form a hexose. The Baeyer scheme of the reaction may be represented as follows:



There is a progressive opening up of the C—O groups, the H atom of the adjacent formaldehyde uniting with the O to form OH. The valence bond of the first carbon atom then may go to the next carbon. This reaction is easily pictured and easily accomplished in the test-tube, but it has been of greater difficulty to plant physiologists than its author ever anticipated. Much time has been spent to demonstrate that formaldehyde exists in plants, and still we are unconvinced. Similarly, many trials have shown that formaldehyde can be used as a source of energy by plants, but still there is a question whether it is so used.

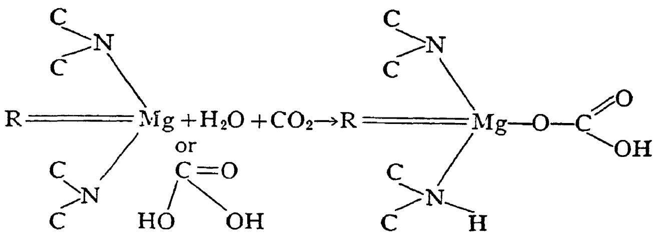
III. *The Synthetic Reactions*

Photosynthesis is dependent upon a rather narrow range of temperature for its action. A Van't Hoff temperature coefficient (Q/10) of 2–3 for the photosynthetic process indicates a rather high degree of complexity for the photosynthetic reactions. This increase of two or three times in the rate of increase of photosynthesis with each 10° C. rise holds only for a limited range, from 0° to 37° C. At low temperatures the Van't Hoff coefficient, Q/10, is higher, and at high temperatures the coefficient is very low, indicating physiological disturbances of very complex nature at these temperatures.

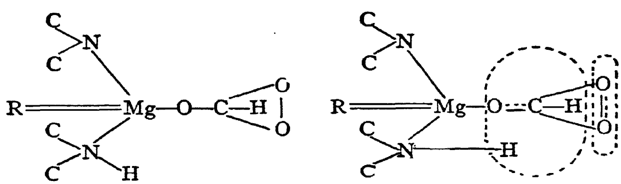
Probably two stages are concerned in photosynthesis, one a photochemical reaction with a low temperature coefficient (1–1.42) and a chemical reaction with a higher temperature coefficient (2.0–3.0).

Chlorophyl in the colloidal state in the plastid can form an additive compound with carbon dioxide similar to the bicarbonates. This is pos-

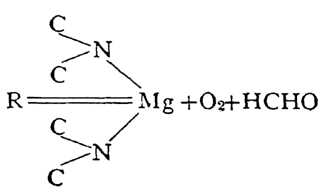
sibly the first step in the photosynthetic reactions. The reactions may be indicated as follows:



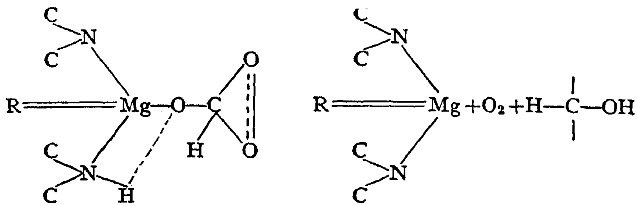
This reaction is reversible, but also a slow decomposition of this compound occurs in the dark to produce $Mg(CO_3)$ in a manner similar to the action of other acids in removing magnesium from chlorophyl to form phæophytin. This intermediate compound then undergoes intramolecular rearrangement, the energy of light is absorbed and a peroxide linkage forms which increases its instability. One oxygen atom is liberated, probably by the action of an enzyme.



The energy absorption increases the instability of this compound which may decompose, producing oxygen and formaldehyde.



Or it will be observed that this reaction might occur in the following steps:



The splitting off of oxygen in the molecular condition seems more reasonable than that atomic oxygen should be split off. The atomic oxygen should be excessively reactive and if produced might combine with oxidizable substances of the cell rather than with another oxygen. The two atoms may be split off simultaneously. They may be joined by one valence already as shown in the formula.

The formation of peroxide-like structures is a property also of other fluorescent pigments. This indicates that the property of fluorescence of chlorophyl may be bound up with a shift in the oxygen linkages.

The activated or nascent formaldehyde $\mathrm{H{-}\overset{|}{\underset{|}{C}}{-}OH}$ has a divalent carbon and has the same composition as formaldehyde, $\mathrm{H{-}\underset{H}{C}{=}O}$. Yet $\mathrm{H{-}\overset{|}{\underset{|}{C}}{-}OH}$ would be more reactive. It is this activated formaldehyde, according to Baly, which might yield the $\mathrm{H{-}\overset{|}{\underset{|}{C}}{-}OH}$ groups of carbohydrates. According to Baly the short ultra-violet wave-lengths about 220 $\mu\mu$ may produce the synthesis of activated formaldehyde from CO_2 and H_2O without the presence of chlorophyl. The polymerization of formaldehyde to hexose is brought about by somewhat longer wave-lengths about 280 $\mu\mu$.

The function of chlorophyl as a photocatalyst in the photosynthetic process is to make an unstable compound with the H_2CO_3. By this combination the frequency of the vibration required to bring about the transformation and storage of energy is made of a longer wave-length than the 220 $\mu\mu$ required for a reaction of the inorganic constituents; carbon dioxide, CO_2, and water, H_2O. The frequency of vibration demanded is brought into the part of the spectrum lying mainly between

the B and C lines (6600—6000 $\mu\mu$). This part of the sun's spectrum has a much higher energy content than the region at the wave-length of 220 $\mu\mu$. The light rays of a wave-length of 220 $\mu\mu$ are largely absorbed from the sun's rays by the atmosphere. The intensity of irradiation at this wave-length is usually very low. Other pigments than chlorophyl, such as malachite green, seem to have a similar effect as photocatalysts in CO_2 reduction by light *in vitro*.

It is well known that reactions involving great energy absorption to drive them in the direction of synthesis require short wave-lengths of light for their photocatalysis. Reactions involving lesser energy change do not require such high-frequency vibrations and may be catalyzed by longer wave-lengths, even down into the heat wave-lengths.

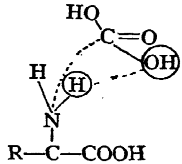
The configuration of a complex molecule, such as the additive compound of chlorophyl with CO_2, is determined by equilibrium between attraction or valence forces which tend to hold it together and instability tensions which tend to disrupt the compound. When the instability tensions exceed the valence forces, decomposition of the compound ensues until a new equilibrium is reached in the system. Definite quantities of energy are absorbed at the decomposition of each molecule. The more closely the complex molecule has already approached its stability limit, the less is the energy needed to be added to decompose it. On the basis of the quantum hypothesis, a definite quantity of light energy is stored in the reaction, and a lower frequency of radiant energy is required when the instability is great. Carbonic acid (H_2CO_3) being a very stable compound is restricted in its photocatalysis to the absorption of light in the ultra-violet region of the spectrum. Any activation which renders carbonic acid sensitive to light of lower frequencies is the result of the production of an increased strain along one of the valence bonds. This increased strain involves the imposition of a reducing potential upon an oxygen of the carbonic acid or the substitution of that oxygen by a reducing agent.

It seems probable that not just a simple additive compound of chlorophyl-a with CO_2 is involved in the photosynthetic reactions, because there exists the oxidized form of chlorophyl-a, which is chlorophyl-b. It has been indicated that in chlorophyl-a one of the acid groups may be bound up in a lactam ring Mg—NHCO. Possibly this group becomes oxidized to a COOH group in chlorophyl-b. The relatively constant proportions of chlorophyl-a to chlorophyl-b indicates that some sort of equilibrium is established between these two compounds. Reactions have been outlined which indicate that the oxygen evolved in photosynthesis may be liberated during the re-formation of chlorophyl-a from chlorophyl-b.

Illuminated solutions of chlorophyl become increasingly metastable, resulting in either a coagulation of the particles in colloidal solution or actual chemical decomposition of the molecule. More should be known about the decomposition products when chlorophyl is exposed to light. Formaldehyde has been demonstrated as one of the products. This has led many chemists following Baeyer to assert that formaldehyde is produced by the photosynthesis of plants; but other explanations, such as the suggestion that activated formaldehyde is the compound, are possible and avoid certain difficulties met with in the Baeyer hypothesis. Such unsaturated compounds, containing a divalent carbon atom as in activated formaldehyde, are highly reactive. The known tendency of substances with the aldehyde grouping to condense may be due to the ease of shifting from the $C{=}O$ to the $C{-}OH$ group. It may be that the chlorophyl molecules in the colloidal condition may be held together by accessory valences of magnesium or nitrogen and thus bring into adjacent positions the newly formed $\mathrm{H{-}\overset{|}{\underset{|}{C}}{-}OH}$ groups. Furthermore it is known that organic substances on activation by X-rays have a regular habit of condensing in a certain-sized polymer, such as acetylene, polymerizing to a solid under the action of X-rays. Probably similar tendency is shown by the activated formaldehyde whereby it tends to produce 3, 6, or 12 carbon units. Bovie suggests that the H_2CO_3 molecule is oriented at the chloroplast surface so as to facilitate condensation.

There is much greater absorption of CO_2 by the leaf than can be accounted for by the combination of CO_2 with chlorophyl. There must be other substances in the cells capable of combining with the CO_2.

The theory of Siegfried that CO_2 is not reduced as such in the plant, but that CO_2 combines first with amino acids and that these carbamino acids enter the chlorophyl reduction complex, has gained some support. Calcium salts of the carbamino acids of glycocoll and alanine were subjected to rays from a quartz mercury-vapor lamp. The illumination resulted in the appearance of slight traces of ammonia, formaldehyde, and methyl alcohol. The conversion of amino acids to calcium carbaminates renders them more sensitive to photo-oxidation, but does not facilitate the reduction of bound H_2CO_3 to formaldehyde or its equivalent. The carbaminate might condense with chlorophyl as well as carbonic acid. The amino acids may function in the photosynthetic process by increasing the absorption and binding of CO_2 by the protoplast. The amino acid concerned may be combined with other amino acids into proteins.



Combination of carbonic acid with an amino acid

If amino acids are of importance in photosynthetic reductions, it would fit in with the idea that the proteins of the plastid are not entirely passive in photosynthesis.

If activated formaldehyde with a divalent carbon is produced, it is possible that photosynthesis results not in the formation of one single compound, but that several final products may be formed, depending upon the physical conditions and upon the supply of accessory substances in photosynthesis, such as the nitrates.

Other constituents of the plastid than the amino acids or proteins may be of importance in the photosynthetic reactions. Chloroplasts are rich in iron. Potassium is usually found abundantly in the chloroplast. The occurrence of potassium around the plastid led Stoklasa to believe that it is of importance in photosynthesis. Potassium is a radioactive element and seems to be necessary for carbohydrate synthesis. Plants with deficient potassium show a bronzed-green color and poor carbohydrate synthesis, indicating some disturbance of the photosynthetic mechanism.

Potassium might accumulate around the plastid through the removal of the NO_3 radical for amino acid synthesis from potassium nitrate, $KNO_3 + H_2O \rightarrow -NH_2 + K^+ + O_2$. Potassium in excess would produce an alkaline reaction in the plastid or around it. A slight shift in the actual acidity (pH) to the alkaline side of neutrality would greatly favor the absorption of carbon dioxide. Such an action could hardly be favored by calcium or magnesium in substitution for potassium on account of their removal as calcium or magnesium carbonate in a slightly alkaline medium.

A deficiency of magnesium in the culture medium of plants has a depressing effect on photosynthesis, probably because the quantity of chlorophyl is decreased. Similarly the lack of iron leads to a decreased photosynthesis, and probably for the same reason.

The photosynthetic process is very easily upset by deficiency of water or of oxygen or by the presence of anesthetics such as chloroform, although the conditions imposed are not sufficient to stop respiration. Photosynthesis will not start in an atmosphere devoid of free oxygen. Evidently

conditions suitable for the existence of the plant by anaërobic respiration are not suitable for photosynthesis. Leaves with a poor carbohydrate supply, such as partly etiolated leaves, cannot withstand as low oxygen concentration and still carry on photosynthesis as those which are well supplied with carbohydrates. Photosynthesis is probably a more complex process than respiration, or a process which requires the functioning of some process of aërobic respiration.

Six definite stages can be recognized in the photosynthetic process:

1. The physical process of diffusion of CO_2 into the leaf. The temperature coefficient, Q/10, of this process is 1–1.4.
2. Absorption of CO_2 to form H_2CO_3 or the HCO_3^{-} and $CO_3^{=}$ ions which may immediately react with basic groups of the amino acids or proteins to form carbaminates. The temperature coefficient in this stage should be that for chemical reactions, Q/10, 2–3.
3. The formation of an addition compound between chlorophyl and either H_2CO_3 or the carbaminate formed in stage 2. The temperature coefficient of this reaction should be of the order of 2–3.
4. Isomerization of the additive compound formed in stage 3 by the absorption of light energy. A peroxide linkage is formed. This is a physical process mainly and should have a Q/10 of 1–1.4.
5. The cleavage of the isomerized chlorophyl peroxide formed in stage 4 to formaldehyde and oxygen, to regenerate the chlorophyl. The temperature coefficient in this stage is such as to indicate enzymatic action. It is this stage which limits the photosynthetic rate at high light and CO_2 intensity. This stage can proceed in the dark. It has been referred to as the Blackman reaction.
6. The polymerization of the activated formaldehyde or ordinary formaldehyde formed in stage 5. The temperature coefficient of this stage should be of the order for chemical reactions or higher, since it may be brought about by enzymatic action.

IV. *Effect of External Conditions on the Rate of Photosynthesis*

The rate of assimilation of carbon in photosynthesis is not only dependent upon the internal conditions which have already been mentioned but also upon external factors. Chief among the factors of the environment which limit the rate of photosynthesis are the carbon dioxide supply, the intensity and quality of the light incident upon the leaf, the water supply, and the temperature.

Liebig in his statement of the Law of the Minimum indicated that growth in the plant was limited by that nutrient which was present in the minimum quantity in proportion to its requirement by the plant. Blackman extended this idea to photosynthesis, involving also environmental conditions other than nutrient substances. Blackman (Fig. 80) states the principle as follows: "When a process is conditioned as to its

rapidity by a number of separate factors, the rate of the process is limited by the pace of the slowest." As an illustration, the following example may be given: Suppose a leaf is exposed to sufficient light and other proper conditions to assimilate 5 c. c. of CO_2 per sq. meter per hour. If it is supplied with 1, 2, 3, 4, 5, 10, 15, 20 c. c. of CO_2, only 5 c. c. of CO_2 will be assimilated and no more. When 1, 2, 3, or 4 c. c. were supplied, the supply of CO_2 limited the rate of assimilation. When 10, 15, and 20 c. c. were supplied, the CO_2 supply was not limiting; light intensity or other factors limited the rate. If the light intensity had been increased, additional amounts of CO_2 might have been assimilated. Blackman points out that other factors than those under observation and control may become limiting factors. We have seen that internal conditions such as the quantity of chlorophyl will influence the rate. The photosynthetic rate is decreased also by deficient water supply. The relation of the interaction of two limiting factors in photosynthesis may be illustrated by Fig. 81. Along the curve AB the rate of photosynthesis is limited by the concentration of CO_2, the light intensity being in excess. But from B to C the rate is limited by the light intensity, the CO_2 being in excess so that further additions do not increase the rate of photosynthesis. If the light intensity should be doubled as shown by ABDE, the light intensity does not become limiting until a higher photosynthetic rate is reached, represented by DE. On further increasing the light intensity it may become a limiting factor only at the photosynthetic rate FG.

FIG. 80.—Frederick Frost Blackman.

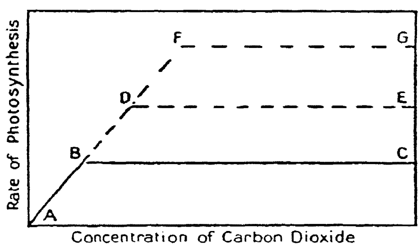


FIG. 81.—Scheme to illustrate the action of a limiting factor. (After F. F. Blackman.)

In similar manner the interaction of light intensity and the temperature may be illustrated by Fig. 82.

Figs. 83 and 84 show the photosynthetic rate when light intensity alone is the limiting factor in the external conditions. At intensities above 3,000 lux the photosynthetic rate does not increase appreciably. Evidently the photosynthetic rate is limited at this intensity by factors internal to the cells.

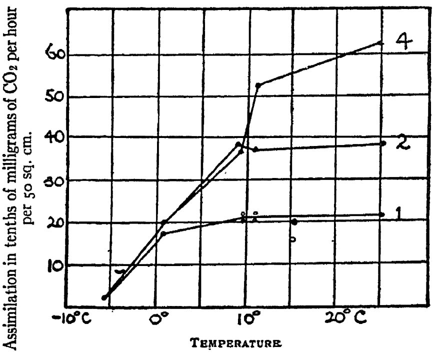


FIG. 82.—Curve illustrating the effect of temperature on assimilation of cherry laurel under the influence of light of different intensities. 1, unit intensity of light; 2, twofold intensity; 4, fourfold intensity. (After Matthaei.)

The effect of concentration of the CO_2 in the water as a limiting factor may be illustrated in the photosynthesis of *Hydrilla* by the graph in Fig. 85. When the CO_2 concentration is above 30 mgs., further increases do not result in an increase in photosynthetic rate. If the graph is exterpolated to cut the horizontal axis, there is indicated a minimum concentration of 1.2 mgs. of CO_2, at which evidently the rate of CO_2 evolution from respiration balances the CO_2 intake from the medium for photosynthesis. At this point the actual photosynthetic rate is made up of the sum of the minimum value and the quantity of CO_2 produced in respiration.

The concentration of CO_2 in the atmosphere as a limiting factor may be illustrated by the graph in Fig. 86. In the free air the content of carbon dioxide is practically constant at three parts in ten thousand of air. But in closed greenhouses even this low value may be decreased unless there is decaying manure to supply carbon dioxide. In forests in which there is much fermentation of organic materials in the soil, the quantity of CO_2 is higher. The CO_2 concentration is quite commonly a limiting factor in the photosynthetic process.

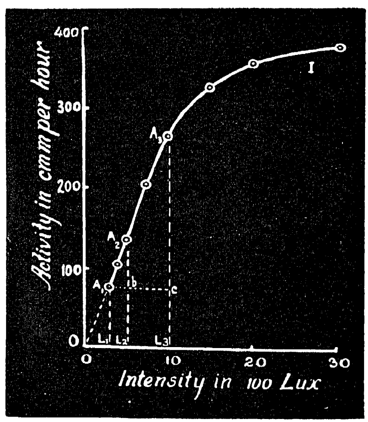


FIG. 83.—Photosynthetic curve under variation of intensity of light. (After Bose.)

The effect of temperature as the only external limiting factor is shown by graph in Fig. 87. The photosynthetic process shows a minimum at

about the temperatures at which the cells of the leaf freeze. From about 15° C. to 27° C. the curve for the photosynthetic rate is nearly

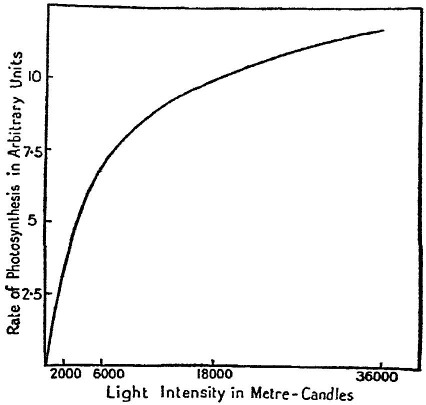


FIG. 84.—Curve to illustrate the relation between light intensity and rate of photosynthesis in *Fontinalis*. (Constructed from the data of Harder.)

a straight-line function of the temperature. There is a sharp maximum which for most plants lies between 30° C. and 35° C., beyond which

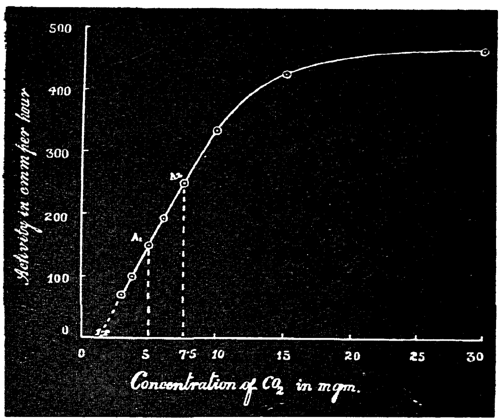


FIG. 85.—Photosynthetic curve under variation of CO_2 concentration. (After Bose.)

there is a rapid falling off in the rate and a second minimum at 47° C. or below.

Kanitz in his *Temperatur und Lebensvorgange* gives the temperature coefficients of photosynthesis as follows (Table 24).

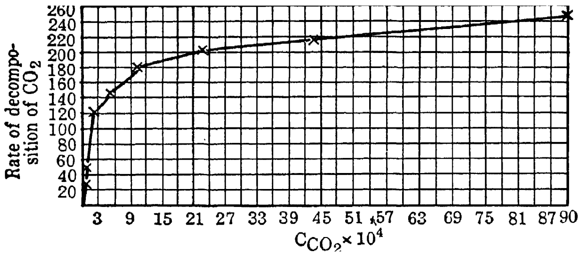


FIG. 86.—Effect of CO_2 concentration on the rate of assimilation in *Chlorella*. (After Warburg.)

TABLE 24

Temperature o°C.	*Assimilation of CO_2*	*Q/10*
−6	.2	
0	1.75	28.7
10	4.2	2.4
20	8.9	2.12
30	15.9	1.76
37	23.8	1.81
40.5	14.0	.23

It should be noted that the value of Q/10 from 0° C. to 37° C. is almost constant. Evidently, within these limits the temperature does not change the rate of its effectiveness, that is, each rise of ten degrees may be relied upon to double the rate of photosynthesis. Below 0° C. the temperature is much more effective than within the range 0° C. to 37° C., and above 37° C. a rise of temperature causes a fall in the coefficient. The maximum of the rate of photosynthesis is reached at 37° C. This is stated as the optimum temperature. Above that temperature, evidently, certain processes which are detrimental to photosynthesis are increased at a more rapid rate than the photosynthetic rate is increased by the rise of temperature. Duclaux gives an excellent picture of the establishment of such an optimum (Fig. 88). If the rate of a process increases with rise of temperature according to the graph AB, processes tending to decrease the effect, as by inactivating the chloroplasts, etc., may be represented by CD.

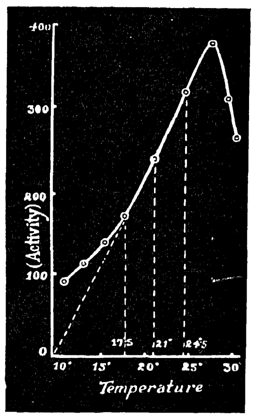


FIG. 87.—Photosynthetic

The result of the interaction of these two opposing reactions will be expressed by a curve AE, which has a point of inflection at the temperature at which the depressing factor overbalances the effect of the stimulating factor. There will result a point at which the process will proceed at the maximum rate. The corresponding temperature is the optimum temperature for the process.

In measuring the effect of temperature upon the photosynthetic rate, the time of exposure to the temperature must be taken into account. In Fig. 89 curve ABC shows the initial photosynthetic rate of cherry laurel. Curve $C_1C_2C_3$ shows the rate two hours after the leaf was placed at the particular temperatures. From the differences in these graphs it is evident that the initial rate of photosynthesis is not maintained, but

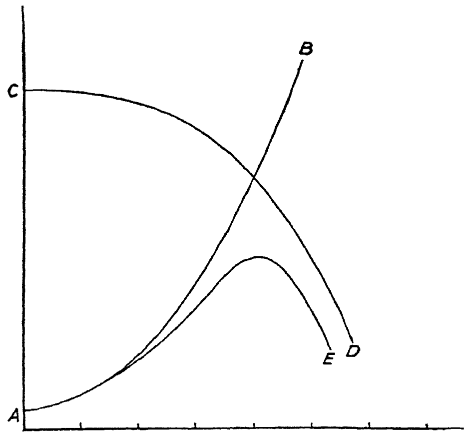


FIG. 88.—Duclaux's explanation of the establishment of an optimum.

a time factor is to be taken into account. The initial rate above about 22° C. falls off with time. The curve ABCDEFG shows the rate at the start of the exposure to the given temperatures C_1, D_1, E_1. If after the lapse of different times of exposure to the temperatures, determinations of the rate are made, it is found that the rates have decreased and are now represented by rates C_2, D_2, E_2, F_2, and later by C_3, D_3, E_3, F_3, C_4, D_4, E_4, F_4, etc. A series of curves can be drawn through the points C_1, C_2, C_3, C_4, C_5, D_1, D_2, D_3, D_4, D_5, and at the higher temperatures these curves may be exterpolated to meet the extension of the curve representing the initial rate curve ABCD. Evidently the maximum temperature at which photosynthesis can occur is determined by the point at which the initial rate of photosynthesis falls immediately to

zero. This may be indicated by the perpendicular line GH. This line strikes the temperature axis at 47° C., which is found to be about the maximum for photosynthesis. In similar manner there could be found a temperature at which the initial rate should be maintained with time, and below which the time factor should not be limiting for the process.

The photosynthetic rate of *Elodea* in water is shown in Fig. 90. A line drawn parallel to the base line cuts the three curves at points which

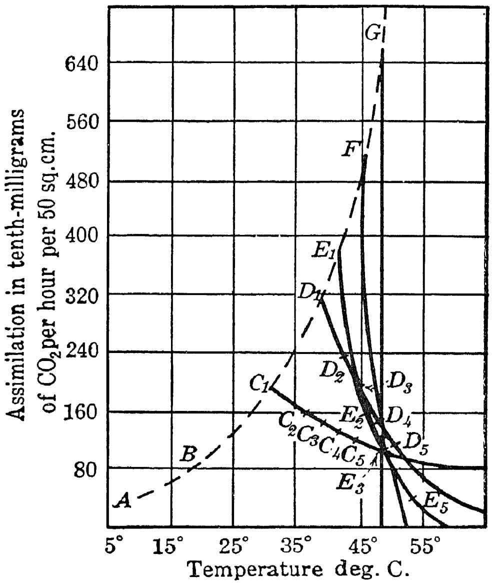


FIG. 89.—Curve showing initial assimilation maxima at different temperatures. For further explanation see text. (After Blackman.)

correspond to the lowest value of each of the three factors necessary to give the rate of photosynthesis indicated on the vertical axis. Thus on line ABC a photosynthetic rate of 0.015 c. c. per hour can be maintained only when the carbon dioxide concentration is not lower than .005%, the temperature not less than 10° C., and the light intensity not less than 4 units. The limiting factor curve may not always adhere rigidly to a typical form with a sharp angle at the point of change of the limiting factor. When two factors are close to the limiting value, a change in the one which is not limiting may have some appreciable effect on photosynthesis. This will show itself near the inflexion point of the curve where the limiting factor is changing.

Under actual conditions of photosynthesis all of the factors undergo fluctuations. But the fluctuations in temperature and light intensity

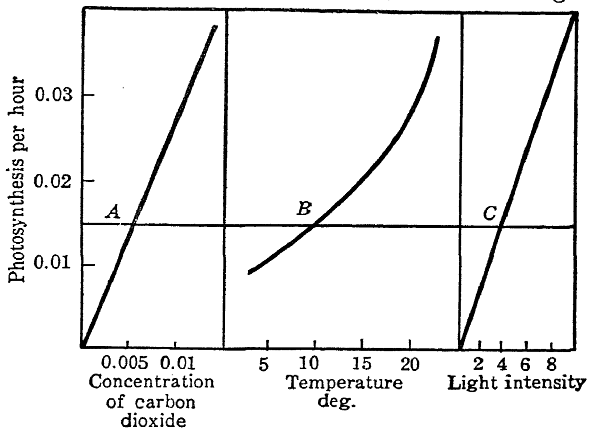


FIG. 90.—Interrelation of environmental factors and rate of photosynthesis in *Elodea*. (After Blackman and Smith.)

show a greater range than the carbon dioxide concentration in the atmosphere. The daily periodicity of temperature fluctuation is a function of the sunlight variation. The light and temperature relationships for a particular day may be represented by Fig 91. The temperature curve

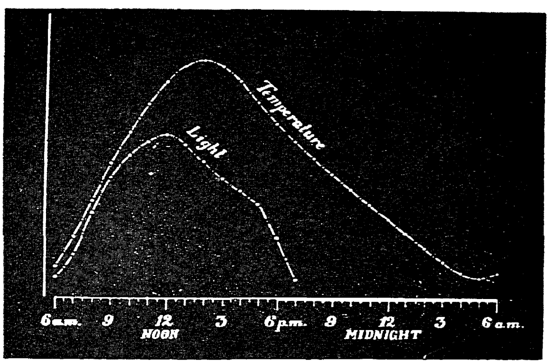


FIG. 91.—Record of diurnal variation of light and of temperature in summer. (After Bose.)

shows an accumulative effect of the sunlight exposure. The light intensity reaches a maximum when the sun is at the zenith, twelve o'clock.

The temperature curve usually does not reach a maximum for two hours after noon.

The length of the light exposure is determined by the position upon

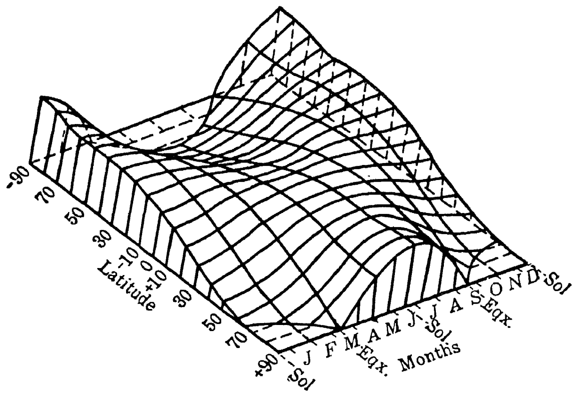


FIG. 92.—Relation of season and latitude to the length of the day.

the earth and by the earth's position in relation to the sun. Fig. 92 gives a summary of the lengths of the day at various latitudes. At the equator

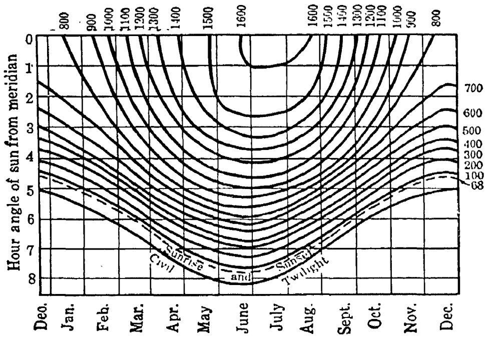


FIG. 93.—Illumination from a cloudless sky on a horizontal surface at latitude 42° north. Foot-candles. (After Kimball.)

the day is twelve hours long; at the poles it varies from zero to twenty-four hours. The angle of incidence of the sun's rays determines the daily range of the light intensity, as shown by Figs. 93 and 94. The light intensity on various planes may be illustrated by comparison

of the intensities of the horizontal and vertical components. The orientation of leaves in different planes determines the light intensity arriving at the surface. The presence of clouds modifies the illumination as shown in Fig. 95.

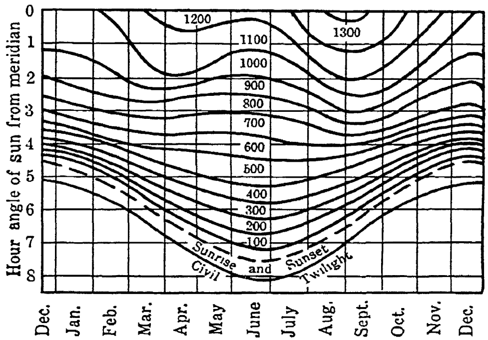


Fig. 94.—Illumination from a cloudless sky on a vertical surface facing south at latitude 42° north. Foot-candles. (After Kimball.)

The position of the sun during the year modifies the illumination on the vertical and horizontal planes so that the total daily illumination

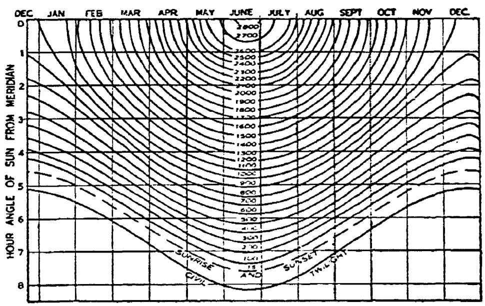


Fig. 95.—Illumination from a cloudy sky on a horizontal surface at latitude 42° north. Foot-candles. (After Kimball.)

varies on these surfaces. When the sun is high (in June for north latitude) the illumination is highest on a horizontal surface (Fig. 96). A vertical surface facing south receives the greatest illumination in February and November (Fig 97).

The fluctuation of the photosynthetic rate throughout the day with the accompanying fluctuation of temperature and sunlight intensity for a particular day, place, and plant may be illustrated by Fig. 98.

The quality of light affects the photosynthetic rate. Only the energy

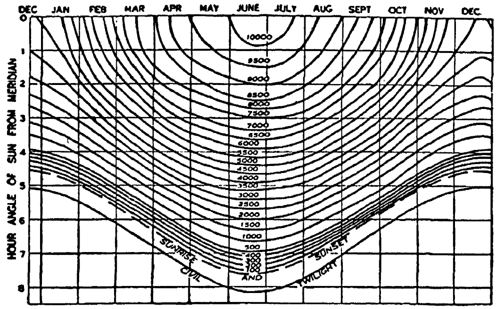


FIG. 96.—Total daylight illumination on a horizontal surface with a cloudless sky at latitude 42° north. Foot-candles. (After Kimball.)

absorbed by the chlorophyl can be used in photosynthesis. The absorption maximum for the green leaf lies between the B and C Fraunhofer lines. The greatest efficiency of the different wave-lengths is at this same wave-length. Fig. 99 shows the energy distribution in the sun's spectrum (thin

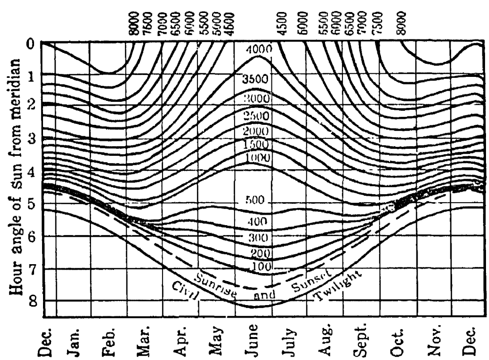


FIG. 97.—Total daylight illumination on a vertical surface facing south with a cloudless sky at latitude 42° north. Foot-candles. (After Kimball.)

line) and the corresponding rate of photosynthesis (heavy line). The energy content of different parts of the spectrum is not equal in this graph. When the energy content at each wave-length is kept the same and only the frequency of the vibration is changed, it is found that the

wave-lengths of light lying between the B and C Fraunhofer lines are most efficient in photosynthesis (Fig. 100). There is a rapid decrease in

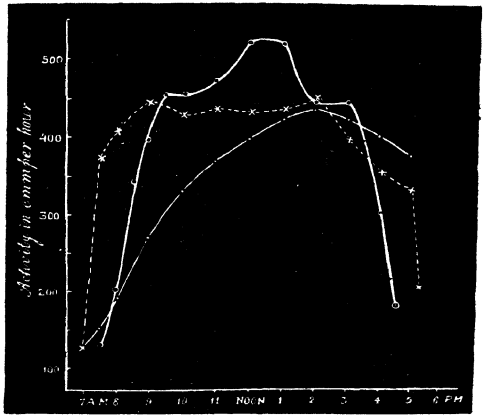


FIG. 98.—The curves for light (dotted line), temperature (thin line), and resultant photosynthetic activity (thick line). (After Bose.)

efficiency for wave-lengths longer than the B line. In the region lying below the B line and marked by the fluorescence color of chlorophyl both

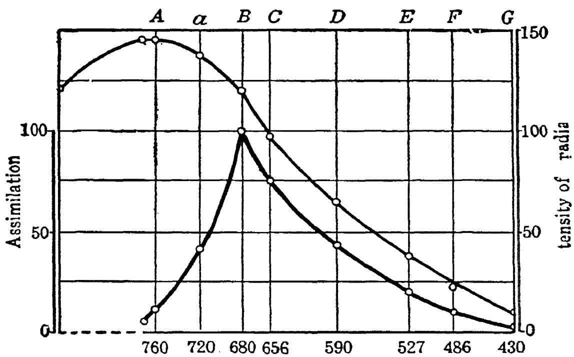


FIG. 99.—Curves showing distribution of energy in the spectrum (thin line) and corresponding photosynthesis (thick line). (After Bose.)

the efficiency and the relative light absorption are less than at other wave-lengths of the visible spectrum. In the violet end of the spectrum the efficiency in photosynthesis is very low, although the light absorption is high. Probably the short wave-lengths are absorbed by other substances

in the leaf than chlorophyl, and for this reason the photosynthetic efficiency is low. Probably the energy absorbed by the carotinoids from the blue-violet region is not available for photosynthesis.

It was reported by Ursprung that plants of *Phaseolus multiflorus* exposed to light cease to form starch after a time owing to "solarization." Leaves exposed for five hours contain abundant deposits of starch, but on nine hours of exposure little starch was present in the leaf. Using

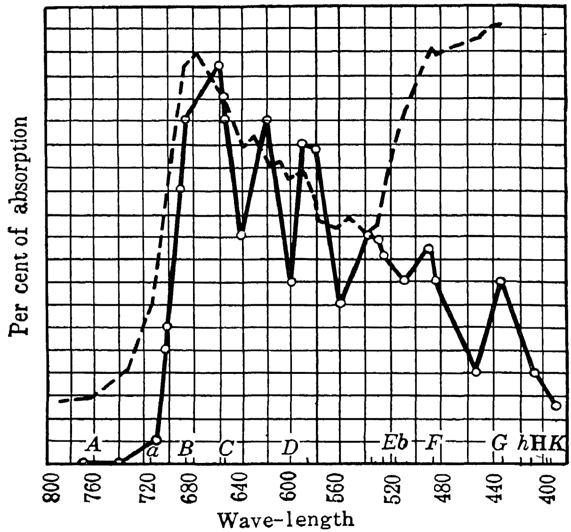


FIG. 100.—Broken line represents the percentage absorption of the light by the green pigments of the living leaf. Solid line represents the starch formation in bean leaf when the energy content of the radiation is the same for each wave-length. (After Ursprung.)

continuous artificial light, however, there is a continuous piling up of starch in the leaves of cabbage until they become richer in starch than potato tubers, the leaves having over 50% acid hydrolyzable materials on the dry weight basis.

With the fluctuating photosynthesis of the day and night alternation, there is a periodic fluctuation of the starch and of sugar concentrations. When the illumination is not interrupted, as under artificial light, the daily fluctuation of carbohydrates ceases. There is established an equilibrium between starch, sucrose, and hexoses, because the conditions are uniform and equilibrium can be reached between the various forms. There will be a balance between photosynthesis, respiration, and growth,

or a balance between the formation and the utilization of the carbohydrates, other conditions in the environment being equalized.

The growth rate is uniform if relatively short intervals, such as succeeding hours or days of an indeterminate growth are measured. The photosynthetic rate may then at the equilibrium point be limited by the accumulation of the photosynthate. Sapozhnikov observed a slowing of the photosynthetic rate after the photosynthate had time to accumulate, and attributed this to the mass action of the accumulated products of photosynthesis. The rate of photosynthesis may be influenced from both ends of the equation, both from the supply of inorganic substances and physical factors and by the rate of removal of the photosynthate.

At a certain intensity of constant continuous artificial illumination the respiratory rate will be just sufficient to balance photosynthesis provided other environmental conditions are constant. This intensity of illumination will be the compensation point (Table 25). There will be neither evolution nor absorption of carbon dioxide, and when it is in excess of the amount used in respiration carbohydrate is available for growth. Using growth as a measure of the compensation point, and with all external conditions constant, Steinbauer found that the minimum light intensity for growth was different for sun-loving and shade-loving species of plants. The light intensity required to maintain the compensation is different for different species of plants, evidently depending upon their efficiency in the use of the light to which they are exposed.

TABLE 25

COMPENSATION POINTS AT ABOUT 20° C.
(Data from Plaetzer)

Species	*Compensation point*
Myriophyllum spicatum	128 lux
Cabomba caroliniana	55
Elodea canadensis (in summer	2
" " (in winter)	18
Spirogyra sp	174
Cladophora sp	253
Fontinalis antipyretica	150
Cinclidotus aquaticus	400

PART VI

PART VI

RESPIRATION

CHAPTER XXVI

MATERIAL AND ENERGY RELATIONS

From its end-result, aërobic respiration can be considered as a reversal of the photosynthetic process. The energy which was stored by the reduction of carbon in photosynthesis is released in respiration by oxidation; but to assume that there is merely a reversal of the reaction, or that the respiratory process represents so simple a reaction as occurs in high-temperature combustion of hexoses, is impossible. Beginning with the usual substrate for oxidation, a hexose, the end-products for aërobic respiration can be represented by the equation $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 674$ L.C. But there are certainly many reactions and intermediate compounds produced in plant respiration. The respiratory process in plants is catalytic. Warburg considers iron, copper, and manganese of great importance as catalysts in respiration. The oxidation of glucose in neutral solution in the test-tube occurs only very slowly at ordinary temperature. Through the catalytic system of the cell, the oxidation becomes rapid. We cannot hope now to outline all of the steps in this process.

I. *The Source of Energy*

Considering the whole plant kingdom, there are many different substances used for oxidation, ranging from simple inorganic substances such as H_2S, NH_3, NO_2, FeO, etc., to the most complex organic substances. In aërobic respiration of higher plants the substance oxidized is most frequently a hexose or some more reactive substance derived from hexoses and having a composition with the same empirical formula. The oxidation of polysaccharides is carried out mostly after cleavage to hexose. The oxidation of fats also may be effected mainly after conversion into hexose. There are many side reactions, such as oxidations of unsaturated fatty acids, which do not require transformation to sugar. The plant oxidizes practically all of the substances which it contains. The utilization and the energy liberated from other substances usually is by no means so great as that from hexoses. From the prominence of hexose oxidations in the plant world, we may believe that even though other substances, such as H_2S, H_2, etc., are the source of energy in the

oxidation of some lower plants, the mechanism in their respiratory processes is that of hexose oxidation. The energy obtained from the oxidation of the inorganic substances is used for the synthesis of organic compounds, which then may be respired. Whatever the source of energy is both in storage and in liberation the energy passes through the hexose stage to a major degree. Certainly from the standpoint of the quantity of energy transformed in the organic world, hexoses are the key substances concerned in respiration.

II. *Emission of Radiant Energy in Respiration*

The energy liberated in oxidation, that is not used in chemical syntheses, finally appears as heat. Heat production in plant parts is a function of the respiratory rate. When the respiration is rapid, radiation to the surroundings from well-insulated plant parts may be insufficient to dissipate the heat produced. The temperature then will rise, and attendant upon this the respiratory rate is increased. There is no mechanism for the control of the respiratory rate, such as exists in higher animals. The temperature of the plant part will rise until thermal emission, transpiration, or conduction is able to dissipate the heat produced, or until the tissue is exhausted at the high temperature. The oxidation of substances in respiration continues even though the temperature is raised by the respiration to a point which does not permit of the further life of the organism. In some cases of fermentations, such as that of hay and sawdust in piles, the temperature actually increases to the ignition point of some of the inflammable products of the bacterial fermentations. Plants under very high oxygen concentration may be killed, evidently by too vigorous oxidation.

By respiration the temperature within closed plum flowers may be raised several degrees above the temperature of the outside air. The respiration of stamens and pistils is rapid. The fertilized ovary respires more rapidly than the unfertilized ovary. The stimulus of the developing embryo is evidently responsible for this difference.

In some fleshy flowers like skunk-cabbage, *Spathyema fœtida*, the floral parts may be kept from freezing by the heat developed in respiration. The temperature within the spadix may be 15° C. above the external temperature. In *Arum italicum* the temperature of the spadices was found to be 51° C. in an air temperature of 15° C.

III. *The Effect of Temperature on Respiration*

At very low temperatures, even in liquid air, there is some evolution of CO_2 by seeds or other tissues which can be cooled to this degree without injury. As the temperature is lowered, the respiratory rate falls off rapidly at about 0° C. (Fig. 101). Between about 5° C. and 40° C. the tem-

perature coefficient Q/10 of respiration lies between 2 and 3. A survey of the whole plant kingdom would show very different temperature relations for the respiration of different plants. Among bacteria and fungi the effect of temperature on respiration is especially variable. The amount of CO_2 evolved gradually increases with the temperature until the plant

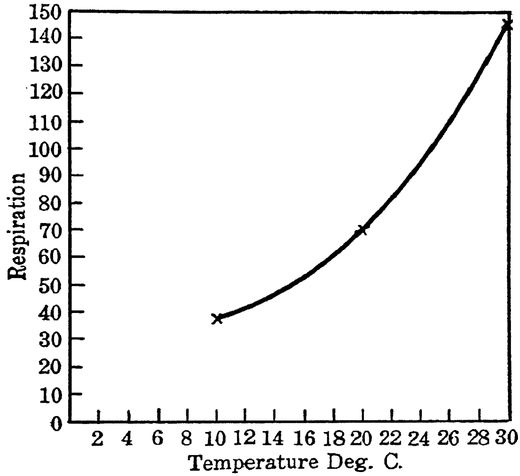


FIG. 101.—Relation of temperature to respiratory rate in *Chlorella*. (After Warburg.)

is exhausted or up to the killing point of the protoplasm, usually at about 50° C. (Table 26).

TABLE 26

CARBON DIOXIDE PRODUCTION BY 100 GMS. OF WHEAT SEEDLINGS PER HOUR IN THE DARK

Temperature °C	CO_2 *in mgs.*
0	10.14
5	18.78
10	28.95
15	45.10
20	61.80
25	86.92
30	100.76
35	108.12
40	109.90
45	95.76
50	63.96
55	10.65

Consequently there is no optimum temperature shown in respiration. There is no effective means of stopping the respiratory rate as the temperature rises. However, the nature of the respiratory process is remarkably affected by different temperatures.

In curves showing the effect of temperature on respiration there is usually found a break in the curve at about 15° C. This same temperature is found to be the limiting value for the oxidation of organic acids. The differences in the slope of the curve above and below about 15° C. may be explained on the basis of the velocities of two catenary reactions such as $A \underset{K_1}{\rightarrow} B \underset{K_2}{\rightarrow} C$, with velocities for the two phases, K_1 and K_2. These two reactions may be differently affected by temperature, since they may be

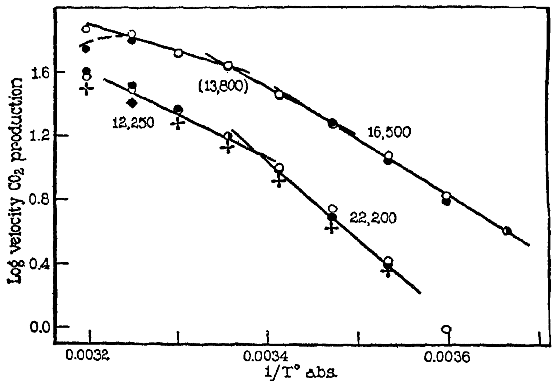


FIG. 102—Upper curve, measurements by Kuijper (1910) of CO_2 production by *Pisum;* white circles, 1st hour, black circles, 2nd hour of exposure to designated temperature. Lower curve, data by Slator on the velocity of CO_2 production by three types of yeast designated by different symbols. (After Crozier.)

catalyzed by different agents. They may show different critical thermal increments in the two phases of the reaction system. The rate of the slowest reaction will determine the rate of the whole process. It follows, then, in a catenary or other complex system in which several reactions are involved, that the critical thermal increment may be different above and below a certain temperature (Fig. 102).

The critical thermal increments (see page 329) calculated for respiratory processes in plants give commonly two values: $\mu = 11{,}500$ and $\mu = 16{,}111$ or 16,700 (Table 27). The first value is encountered at temperatures above 15° C., the second below that temperature. For the reduction of methylene blue (Fig. 103) by bacteria through the removal of H from succinic acid, $\mu = 16{,}700$. This value is common for dehydrogenation

TABLE 27

CRITICAL THERMAL INCREMENTS OF RESPIRATION

Object	Temperature range °C.	Critical temperature (when evident) °C.	Lower tempera-tures	Higher tempera-tures
Arbacia eggs (O_2 utilization)	3 to 31	14, and 25	11,800	16,140
Mytilus gill epithelium (O_2 utilization)	1 to 34.5	15	16,700	11,590
Goldfish (O_2 consumption)	0 to 28		16,100	
Toad (O_2 utilization)			16,800	
Pisum (CO_2 production)	0 to 26		16,200	
Yeast (CO_2 production)	5 to 40	22.5	22,200	12,250
Ulva (CO_2 production)	17 to 27			12,400
Pisum (CO_2 assimilation)	6 to 37	16.0	12,920	10,290
Ulva (CO_2 assimilation)	17 to 27			10,300

mechanisms of widespread occurrence and is probably indicative of iron catalysis. The value of $\mu = 11{,}500$ is indicative of the catalytic action of OH^- in respiration. The value $\mu = 16{,}140$ is associated with the oxidation of Fe'' and may be compared, first, with that of respiration in sea-urchin eggs for which iron is catalyst, and, second, with that for some simple reactions in which Fe is known to serve as catalyst. This value of the critical thermal increment is not found for oxidative reactions in

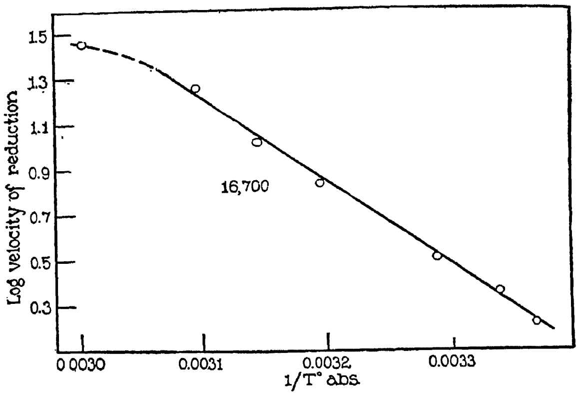


FIG. 103.—Critical thermal increment for the reduction of methylene blue by bacteria, succinic acid $\rightarrow$ methylene blue $\rightleftarrows$ fumaric acid + leucomethylene blue. Above 50° C. destructive effects of temperature become evident. (After Crozier.)

which Fe is not involved as a catalyst. The fundamental importance of iron as a catalyst is indicated by this relation.

IV. *Light Production*

In some oxidation processes the energy liberated may appear as light, that is, as a much shorter wave-length than heat. This phenomenon of light emission on oxidation is shown by a number of easily oxidizable substances and is known as *phosphorescence* or *bioluminescence*. Bioluminescence may be produced by a substance luciferin on oxidation by an oxidizing enzyme luciferase. The phosphorescent substance, such as luciferin, is produced in the organism and may be oxidized with light emission after death of the organism. No heat is produced in the oxidation of luciferin by luciferase.

A number of bacteria and fungi show the phenomenon of phosphorescence. Certain wood-rotting fungi are the best known examples, since they may be observed commonly to produce "foxfire" in rotting stumps. Certain species of *Agaricus*, *Polyporus*, and *Auricularia* most commonly

produce phosphorescence. Among bacteria which emit light are certain genera found commonly on putrefying meats, especially on fish. Light emission by these forms is dependent upon abundant oxygen supply.

V. *Respiratory Intensity*

Respiration proceeds during light exposure of green plant parts, but the evolution of CO_2 may be stopped by its use in photosynthesis. Respiration during the day amounts to only a small fraction of the energy stored in photosynthesis at the same time, yet the respiration during insolation may proceed at a more rapid rate than in darkness, firstly because the oxygen supply is greater, and secondly because there is ionization of the atmosphere in sunlight exposure and this increases the rate.

The respiratory rate depends upon the stage of development and the amount of living substance in the tissue. In rapidly growing organs like root and stem tips and in buds, the respiratory rate is scarcely less than in animals. The amount of CO_2 given off per unit time and per unit weight of living substance gives a measure of the respiratory intensity. In plants much of the weight is due to cellulose of the wall, which is physiologically inert, so no accurate comparison can be made except on the basis of the amount of protoplasmic materials. Cork and dead wood do not respire, and in old cells there is proportionally much less living protoplasm than in embryonic cells.

The following table gives the respiratory intensity of some types of plant tissues compared on the basis of 1 gm. dry weight of each tissue. The respiratory rate for bacteria and fungi in general is greater than for higher plants (Table 28).

TABLE 28

	Temp.	*Respiratory intensity in 46 hrs.*
Leaf buds of *Syringa vulgaris*	15° C.	35 c. c. CO_2
Leaf buds of *Ribes nigrum*	15° C.	48 c. c. CO_2
Germinating seeds of *Sinapis nigra*	16° C.	58 c. c. CO_2
Germinating seeds of *Lactuca sativa*	16° C.	82.5 c. c. CO_2
Germinating seeds of *Papaver somniferum*	16° C.	122.0 c. c. CO_2
Azotobacter chroöcoccum	.....	709.5 c. c. CO_2
Aspergillus niger, 2 day culture on quinic acid	.....	1874.0 c. c. CO_2
Aspergillus niger, 3 day culture on quinic acid	.....	682.0 c. c. CO_2
Aspergillus niger, 4 day culture on quinic acid	.....	276.1 c. c. CO_2

The effect of age of the tissue can be seen from comparison of the rates for 2, 3, and 4 day cultures of *Aspergillus*. The rate decreases rap-

idly after the second day in the culture. This organism grows and respires very rapidly. Probably the decrease in respiratory rate with age of the culture is due to staling products which tend to inhibit it. Also, the comparison of respiratory intensity on the basis of total dry weight is not accurate on account of the greater percentage of inactive constituents in older mycelium.

Resting seeds and dormant buds show a comparatively low rate of respiration. Germinating seeds and sprouting buds show a rise of respiratory intensity almost exactly paralleling the curve for the grand period of growth. In root tips and stem tips the respiratory rate is also a function of the curve for the grand period of growth. There is a rapid rise to a maximum, followed by a gradual decrease in rate. The areas most rapidly growing in stem and root show the highest respiratory intensity. They show also the highest activity of oxidase, catalase, and peroxidase. The energy expended by roots in pushing into the soil and for growth comes from the energy liberated in respiration.

Embryonic tissues respire more rapidly than surrounding tissues of the endosperm. Young tissues in general show a rapid respiratory rate. Stamens and anthers of opening flowers respire rapidly.

VI. *Changes in Respiratory Intensity Due to Stimuli*

The respiratory intensity can be greatly modified by various stimuli. Physical stimuli which produce growth or increase protoplasmic activity cause increases in respiratory rate. Chemical stimuli may greatly increase the respiratory rate. The exposure of green bananas to one part of ethylene in one thousand parts of air causes a great increase in respiratory rate. Other anesthetics, such as ether and chloroform, increase the respiratory rate, a phenomenon probably associated with the use of anesthetics to break the rest period.

The effect of the anesthetics on the protoplasm may be to increase the permeability, either increasing the supply of oxygen or permitting the mixing of certain enzymes and their substrates, thus increasing the respiratory materials.

A number of substances which are toxic at higher concentrations stimulate respiration at relatively low concentrations, a few parts per billion of solution. Perhaps this is an explanation of the oligodynamic effect of such substances as copper, arsenic, zinc, etc. At high concentrations such toxic agents immediately depress the respiratory rate. The production of CO_2 in respiration may be entirely suspended by the influence of HCN without the subsequent death of the tissues. Possibly cyanogenetic glucosides may have a function in controlling the respiratory

rate. The cyanides may stop respiration by paralyzing the catalytic action of iron compounds.

The respiratory rate is increased by an increase in alkalinity. Exposure to ammonia fumes increases the respiratory rate, and if the concentration is low may cause growth responses. Slight freezing of tissues stimulates respiration. Wounding, as in cutting tubers of potato, evokes an increased protoplasmic activity and attendant rise in respiratory rate.

A high respiratory rate is maintained by tissues containing a high concentration of respiratory pigments. Plant parts which show quantities of anthocyanin pigments usually have a higher respiratory rate than parts lacking these pigments. The anthocyanins may function as respiratory pigments. Also, areas showing large quantities of anthocyanins are frequently higher in direct reducing substances than adjacent areas which lack anthocyanins.

VII. *Irreversibility of the Respiratory Process*

In general, the only available external source of radiant energy to be stored in plants is light, for a greater frequency of vibration than that of heat is required n reactions involving large energy transfers. When we have the introduction of light energy, we are concerned with photosynthesis alone, for there is fairly good evidence that this is the only process by which energy from physical sources may be stored as chemical energy by plants. At the range of temperatures found in plants, heat catalyzes the synthetic reactions by increasing the molecular instability or reactivity without heat energy being stored in the process as chemical energy. The quanta of energy stored in the synthesis must come from other sources. There is no reversal of the respiratory process at more advanced intermediate stages. All of the intermediate compounds which are produced with great liberation of energy must be fully oxidized to CO_2 before resynthesis to hexoses by the absorption of light energy in photosynthesis can occur. Organic acids, such as citric, malic, or oxalic acids, produced by the partial oxidation of hexoses, are not again reduced to hexoses, but they must undergo complete oxidation to CO_2 before resynthesis to hexoses by photosynthesis can occur. In photosynthesis only carbon in the fully oxidized condition can be used.

There may be resynthesis of hexoses or other slightly more reduced organic compounds from more highly oxidized substances such as lactic acid. Such resyntheses involve mainly polymerization of carbon chains and atomic rearrangements which require but very little energy change. For such small energy changes possibly heat energy may be used in the reaction, for with the decrease in the energy required to reverse the reaction the wave-length of the energy which may be used is brought into

the lower frequency of vibrations. In muscular activity there is such a reversal of the reaction. One-half of the glucose molecule is completely oxidized; the other half forms the lactic acid of muscle. This lactic acid is again resynthesized into glucose, but it is not possible to say that heat energy alone is used even in this case. The energy for resynthesis may be chemical energy.

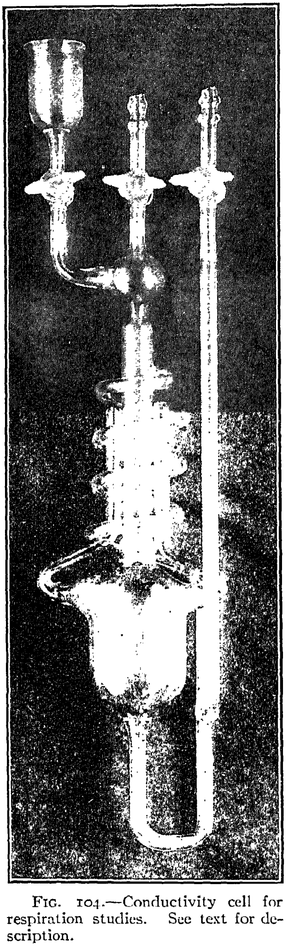


FIG. 104.—Conductivity cell for respiration studies. See text for description.

VIII. *Methods of Measuring the Respiratory Rate*

It is convenient to use the same sample of tissue in studying changes of respiratory rate coincident with modification of the environment. It is desirable to be able to obtain continuous readings of the rate of CO_2 production and at short intervals. The CO_2 produced can be absorbed from a closed system containing the plant material by alkali and weighed directly or titrated if the CO_2 output is large. For small amounts of CO_2 the error due to CO_2 absorption from the atmosphere is not negligible. In such cases it is convenient to use conductivity methods, determining the CO_2 produced by changes in the conductivity of $Ba(OH)_2$ solutions in an absorption cell (Fig. 104).

The gas enters by the long tube at the right, rises through the spiral in interrupted bubbles mixed with the $Ba(OH)_2$ solution, and passes out the center tube, which is provided with a bulb to break up the films. The absorbing solution is thoroughly mixed and continually circulated through the spiral. The funnel side-tube is useful for filling the apparatus. Connections to the electrodes are made through glass tubes at the sides. The cell is made of pyrex glass and the change of the capacity factor is found to be negligible since each electrode is protected from the deposition of $BaCO_3$ on it by a projecting glass collar. The electrode vessel is filled with a known volume of $Ba(OH)_2$ solution (100 c. c.) so that the level

stands below the top of the spiral when the cell is in operation. The concentration of the $Ba(OH)_2$ solution may be varied within the limits of the capacity of the electrodes. Usually n/20 to n/200 $Ba(OH)_2$ is used. The cell is arranged for immersion in a water thermostat.

IX. *The Source of Oxygen for Respiration*

The source of oxygen for plant respiration may be a great variety of compounds containing oxygen, but with regard to quantity, the oxygen of the air is most used. This is equivalent to the statement that plant respiration is mainly aërobic, and that is probably for the reason that most plants live under aërobic conditions. However, stages in the respiration of higher plants even under aërobic conditions may be considered as anaërobic or intramolecular respiration because the oxidation of one atom of carbon may be affected by oxygen derived from adjacent atoms in the same molecule or from other molecules. The source of oxygen for many bacteria and fungi which live in places where oxygen penetration is slow is from combined oxygen, either the oxygen of nitrates, sulphates, water, or other oxidized inorganic substances, or from the oxygen contained in organic substances. Such organisms are either facultative or obligate anaërobes. In the use of oxygen from such sources it must be possible to derive more energy by the oxidation of the oxygen acceptor than is concerned in the reduction of the oxygen donator. Otherwise energy must be added from some external source to make the reaction proceed.

Where the energy liberated by oxidation of the oxygen acceptor is greater than that required for the reduction of the oxygen donator, even if intermediate stages to the contrary are interposed, the reaction will proceed and the energy required for the activities of the plant may be gained in this manner.

There is no mechanism in plants whereby molecular oxygen can be evolved except in the photosynthetic reactions. Only when oxygen is taken on by a nascent highly reduced part of some molecule which is undergoing decomposition, or when it is taken on by another oxygen acceptor with a higher reduction potential than exists in the substrate concerned, is oxygen removed from the organic compounds of the plant. All processes in the cell other than photosynthesis tend to liberate energy, and if one atom is reduced, another must be oxidized, with equal or a greater liberation of energy than was required for the reduction. The highly reactive, nascent fragments of hexoses and other organic substances in cells, probably due to their unsaturated valences, may combine with oxygen to produce a more fully reduced condition than that of the original substrate, but this action is always accompanied by an

attendant equal or greater oxidation of the other fragment or molecule to liberate the energy. Alcohols may be produced from the corresponding aldehydes if there is simultaneously an oxidation of another molecule of aldehyde to the corresponding acid to supply the energy. This is a simultaneous oxidation and reduction first demonstrated by Cannizzaro (Fig. 105), and known as the *Cannizzaro reaction.* This reaction may be catalyzed by the Schardinger enzyme, an oxydoreductase. There is a question if such energy exchange ever involves heat production or absorption. It seems more reasonable to consider that chemical energy only is involved in the exchange of electrons. In all reactions of the Cannizzaro reaction type an oxygen acceptor is required, that is, the oxygen is combined into another molecule having less energy content than the original compound, or there may be a hydrogen donator which combine with the oxygen of the oxidized organic substance, reducing it and combining with the oxygen to form water. The oxidation of hydrogen by oxygen liberates a great amount of energy which may then be consumed partly in the reduction. In the reduction of nitrates or sulphates the oxygen goes to form more stable compounds, more stable because they contain less energy. Nitrates may be used more easily than sulphates because less energy is required to reduce them to nitrites or to ammonia than is required for the reduction of H_2SO_4 to H_2S. Consequently, the efficiency of oxidations using the oxygen of nitrates is relatively high. For the oxidation of tartaric acid with the complete reduction of nitrate to atmospheric nitrogen, the energy loss is about 90% of the energy liberated from the oxidation of tartaric acid by free oxygen.

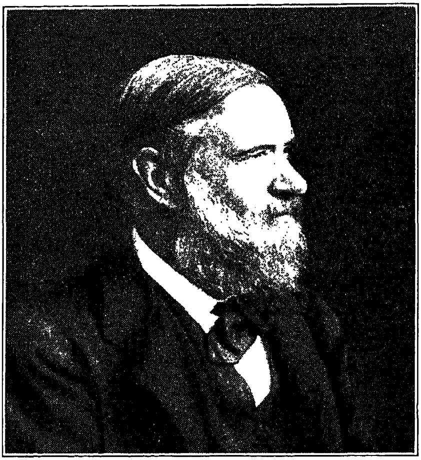


FIG. 105.—Stanislao Cannizzaro, 1826–1910.

cal.

$$2C_4H_6O_6 + 4HNO_3 \rightarrow 8CO_2 + 8H_2O + 2N_2 + 506.8 \text{ cal.}$$

$$\frac{506.8}{564} = 89.8\%$$

For the oxidation of glucose with the oxygen of sulphates, the energy liberation is only about 10% of that liberated in complete oxidation of glucose with atmospheric oxygen.

$$3H_2SO_4 + 610 \text{ L.C.} \rightarrow H_2S + 6O_2$$
$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 674 \text{ L.C.} \quad 674 - 610 = 64 \text{ L.C. liberated}$$

In the formation of butyric acid in the anaërobic oxidations of some putrefactive bacteria, hydrogen is evolved. The butyric acid molecule is a highly reduced substance. The substances formed in the reaction under highly reducing conditions evidently liberate but little energy, for nearly all of the energy liberated in the formation of CO_2 must be consumed in producing the accompanying highly reduced products. $C_6H_{12}O_6 \rightarrow 2CO_2 + 2H_2 + C_3H_7COOH + 15.6$ cal. The efficiency of the reactions represents only about 2% of the total energy liberated by glucose on complete oxidation.

X. *Oxygen Supply to the Tissues*

When the stomata are open there is free passage for oxygen by diffusion through them. When the stomata are closed the oxygen supply is decreased, but not to so great a degree as the transpiration is decreased, because oxygen can pass through the cutinized epidermal cells more easily than water can.

Oxygen of the air enters the cell in solution in the water which imbibes the wall. In plants which are submerged in water, the oxygen supply is dependent upon the solubility of oxygen in the water and the transfer of this downward through mixing currents. Probably the rate of oxygen supply to such plants may be somewhat deficient during the night. Many water plants are provided with air chambers within hollow stems which facilitate the diffusion of the oxygen produced in photosynthesis and which also act as oxygen reservoirs for the respiration of the stem tissues at night. That oxygen evolved from the leaves is conducted regularly into these channels can be demonstrated by pinching off a sprig of *Elodea* and inverting it in a vessel of rain-water in the sunlight. Bubbles quickly begin to come off in a stream from the end where the stem was pinched off. The quantities of oxygen produced in photosynthesis ensure an abundant oxygen supply for leaves, and by diffusion from them for adjacent stem tissues. Many stems have layers of photosynthesizing cells in the young bark. These cells by photosynthesis increase the oxygen supply of the stem. Stems are provided with stomata in very young epidermis or with lenticels in the young bark which serve to facilitate the diffusion of oxygen into the stem tissues. The tracheæ of large trunks of trees supply passages for gas diffusion, but there is evidence that the

oxygen supply into the interior of large stems is somewhat deficient. Frequently there are deposits of oxalates produced by incomplete oxidation of carbohydrates in stems. The oxygen supply from the soil to roots in many habitats may be deficient. In bogs the high concentration of organic matter undergoing oxidation decreases the oxygen available to plant roots. When the soil contains a high percentage of clay which may pack or bake into impermeable layers, there are shown evidences of oxygen starvation by the roots. Where tree roots are covered over with concrete sidewalks, the tree may be killed by oxygen starvation of the roots. Some trees are much more sensitive in this regard than others. Probably this is related to the ease with which oxygen may be conducted from the parts above ground. Many trees die when the roots are buried too deeply in the soil by natural or artificial means. Some have the ability to throw out new roots from the stem in time to support the growth of the top.

Soil which has a fairly high content of organic matter may be so low in oxygen content as to prevent the germination of some seeds when they lie at a depth greater than about eight inches. The oxygen-supplying power of soils varies greatly with soil texture and composition. Sandy soils or soils which are made loose by tillage afford better oxygen penetration than packed clay soils. Soils rich in organic matter, especially when green manure is plowed under, are liable to show oxygen deficiency.

Fruits sometimes have stomata or lenticels in the epidermis which facilitate oxygen movement into the deeper layers of tissue. But the oxygen supply to thick layers of tissue is insufficient. Organic acids are frequently produced in fruits from incomplete oxidation of carbohydrates. Fruits which possess green pigments in their surface layers may obtain considerable quantities of oxygen by the photosynthesis going on in these layers in daylight. In ripening fruits there is frequently a breaking apart of the cells which makes the intercellular spaces larger and facilitates oxygen diffusion. The stem scars in tomatoes and other fruits allow ready diffusion of gases.

When the supply of oxygen is limited, the conditions within the tissue become more highly reducing. There are left in the protoplasm organic acids and other substances which are not so easily oxidizable as the original substrate, the hexoses. The limited oxygen supply is consumed by union with the more highly reducing substances of the cell. The accumulated products of incomplete respiration may be further oxidized when the reduction potential is decreased by the introduction of oxygen. Thus in cacti and other succulent tissues which have a thick impermeable cuticle, the oxygen supply at night is insufficient and organic acids are formed from carbohydrates. These increase during the night in *Opuntia*,

so that by early morning the juice has a distinctly sour taste. When the plants are exposed to sunlight in the morning, photosynthesis begins, and this so increases the oxygen supply and the oxidation potential that the organic acids may be completely oxidized to CO_2 and H_2O.

XI. *Respiratory Ratio*

When the substrate undergoing oxidation by oxygen of the air has the empirical formula $(CH_2O)_n$, that is, when the hydrogen and oxygen in the compound are in the proportions to form water, the volume of the oxygen required will be equal to the volume of CO_2 produced in respiration, because equal numbers of gram-molecular weights of the reactants correspond to equal multiples of the gram-molecular volume: 22.4 liters.

$$(CH_2O)_n + nO_2 \rightarrow nCO_2 + nH_2O$$
$$n \times 22.4 \text{ L.} \rightarrow n \times 22.4 \text{ L.}$$

The ratio of the volume of CO_2 produced to the volume of O_2 consumed is known as the *respiratory ratio*. In the above equation for the oxidation of a substance of the empirical formula $(CH_2O)_n$, the ratio is equal to unity $\frac{CO_2}{O_2} = n/n = 1/1$.

With atmospheric oxygen, the respiratory ratio will not be changed from this value as long as the substrate and the oxidation products are the same, although many intermediate products may be formed and provided the concentration of any intermediate substances does not change. For example, let us suppose that starch is the substrate undergoing complete oxidation. Although the starch must be split to glucose and then carried through a series of intermediate compounds, the respiratory ratio will still be unity. If a fat, triolein, is undergoing complete oxidation, it may undergo hydrolysis, the glycerin may be oxidized in one series of changes and the fatty acid cleaved and transformed into glucose and then oxidized in another series of changes; but the respiratory ratio will not change as long as the concentrations of the intermediate compounds do not increase or decrease. But if for any reason there is an accumulation of any intermediate, the ratio will change from its value for the complete oxidation of triolein. Thus in the transformation of fat into carbohydrate, oxygen is consumed but no CO_2 may be evolved.

For the purpose of finding the respiratory ratio, we need not be concerned with the intermediate compounds or the probable course of the whole series of reactions; but we may write equations and use the quantities of materials involved as they would be required in complete combustion at high temperature. The respiratory ratio is then a valuable

physiological tool for indicating the nature of the substance being oxidized. It is especially useful in case one substance principally is undergoing oxidation. Thus in a medium containing cellulose and oxalic acid, the respiratory ratio would give an indication of which substance was being respired by a fungus growing in the medium.

The respiratory ratio for the complete oxidation of all carbohydrates and all substances of the same empirical formula $(CH_2O)_n$ as carbohydrates is unity.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

$$\frac{6 \text{ molecular}}{\text{volumes}} = \frac{6 \text{ molecular}}{\text{volumes}}$$

$$CH_3COOH + 2O_2 \rightarrow 2CO_2 + 2H_2O$$

The respiratory ratio for the complete oxidation of fats is generally less than unity. More oxygen must be absorbed than carbon dioxide is evolved, because part of the oxygen is used for the oxidation of hydrogen which is in excess of the quantity required to form water. The respiratory ratio for the complete oxidation of tristearin may be found as follows:

$$C_{57}H_{110}O_6 + 163O_2 \rightarrow 57CO_2 + 55H_2O$$

$$\frac{CO_2}{O_2} = \frac{57}{163} = 0.35$$

The respiratory ratio is generally less than unity for the complete oxidation of substances containing hydrogen in excess of twice the number of oxygens present, and it will deviate from unity in proportion to the excess of hydrogen. This is true also for substances which contain other atoms than carbon or hydrogen, which may combine with oxygen to reduce the proportion of $\frac{CO_2}{O_2}$.

The complete oxidation of the organic acids having the hydrogen present in less proportion than that required to form water produces volumes of CO_2 greater than the volumes of oxygen required. The respiratory ratio for such acids is greater than unity.

$$2HCOOH + O_2 \rightarrow 2CO_2 + 2H_2O$$

$$\text{ratio } \frac{CO_2}{O_2} = 2/1 = 2.0$$

$$2(COOH)_2 + O_2 \rightarrow 4CO_2 + 2H_2O$$

$$\frac{CO_2}{O_2} = 4/1 = 4.0$$

In cases of the oxidation of one molecule or part of a molecule by oxygen already present in the compounds of the plant, there may be no oxygen absorption, and the denominator of the fraction expressing the respiratory ratio falls to zero. Thus for the fermentation of glucose by zymase action, the reaction may be represented as follows:

$$I_3CH_2OH$$

$$2/0 = \infty$$

In the case of the oxidation of hexose to oxalic acid or other organic acids, the respiratory ratio may be very low. There may be a considerable absorption of oxygen, but the oxidation does not proceed as far as to gaseous CO_2. When the highly oxidized product is completely oxidized, a little oxygen absorption will produce large amounts of carbon dioxide.

XII. *Conditions Affecting the Nature of the Oxidation*

The nature of the substance undergoing oxidation in the plant is determined by the temperature, by the oxygen supply, and by the internal conditions of the plant.

In general, temperatures from 15° C. to 40° C. favor complete oxidations where the oxygen supply is sufficient. Organic acids and other products of incomplete oxidation may be fully oxidized by elevating the temperature. But even if the oxygen supply is in excess of the demands, at low temperatures (−5° C. to 15° C.) there may be an accumulation of incomplete oxidation products in the case of some tropical and subtropical plants such as bananas. If the fruits are held at too low temperature in storage, they are injured more than at intermediate temperatures. Evidently the products of respiration at low temperatures are toxic.

In the respiration of the grape during ripening, two principal classes of substances are the substrates, hexoses and organic acids, chiefly tartaric acid. At temperatures below about 15° C. there is no oxidation of tartaric acid. The respiratory ratio is then about equal to unity because sugars are undergoing oxidation. When the temperature is raised above 15° C., there is a change in the respiratory ratio to a value markedly above unity $\frac{CO_2}{O_2} > 1$. This is for the reason that the tartaric acid, rich in oxygen, is being oxidized.

In green apples, owing to the influx of substances from the tree, the concentration of starch, sucrose, and reducing sugars continually in-

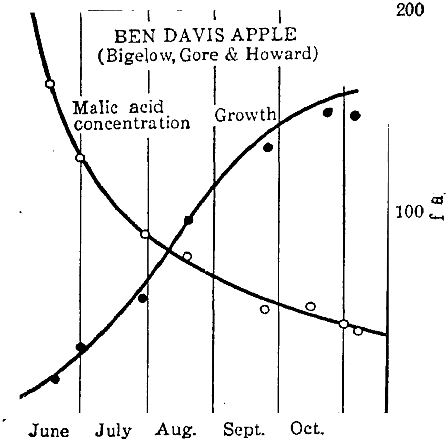


FIG. 106.—Malic acid content of Ben Davis apple at various stages of growth. (After Kidd and West.)

creases. Fig. 106 shows the concentration of malic acid in an apple at different stages of growth. The acidity decreases with age. Fig. 107 shows the respiratory rate of an apple at different stages in its growth. The respiratory rate of young fruits is very high, with a rapid decrease in rate as the apple grows. There is a close parallel between the rapidly falling respiratory rate and the rate of decrease of malic acid. The respiratory rate in the growth phase of apples seems to be controlled by the concentration of malic acid. Near the time of maturity the respiratory rate falls to a practically constant level, and the malic acid concentration remains about constant. The falling off of the respiratory rate is largely due to the exhaustion of the malic acid in respiration. The respiratory ratio changes from 1.2 to 0.86, giving clear evidence of the change in the nature of the materials undergoing combustion. The rate of loss of malic acid in apples stored at 1° C. increases with the time of storage, while the rate of loss of sugar decreases (Fig. 108).

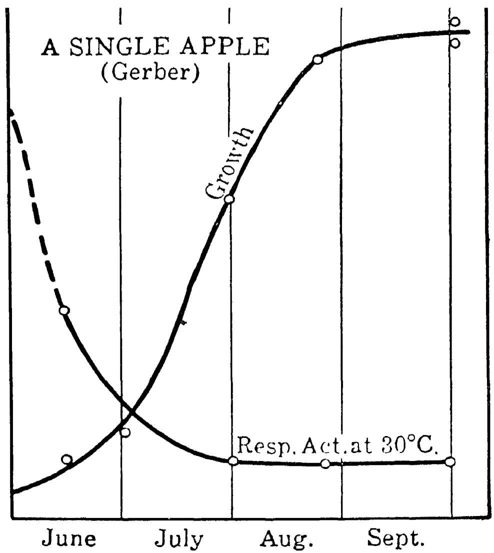


FIG. 107.—The rate of growth and the respiration rate of an apple.

In the green Japanese persimmon there are large quantities of tannins as well as carbohydrates, but little organic acids. Raising the temperature does not appreciably change the respiratory ratio of green persimmons. In later stages there is an increase in the respiratory ratio. This is caused by the production of mucilages in the ripening fruits, which block the intercellular spaces and inhibit oxygen diffusion. Owing to the oxygen deficit, intramolecular respiration increases the respiratory ratio to a value greater than unity. The high ratio here is due to lack of sufficient oxygen. Under these conditions the sugars may be oxidized to produce ethyl alcohol and higher alcohols, organic acids and esters which produce the aroma of the ripe fruits. The flavor of some persimmons is improved by the restricted oxygen supply when the fruits are ripened in closed vessels, owing to the production of organic acids which relieve the "flat" taste of fruits having little acidity. Similar conditions are found in the ripening of bananas, melons, and other fruits. If the oxygen supply for ripening bananas is deficient, there is a production of a slight acidity and more aromatic substances are produced in the fruits. In some varieties of bananas the production of slight acidity may improve the flavor.

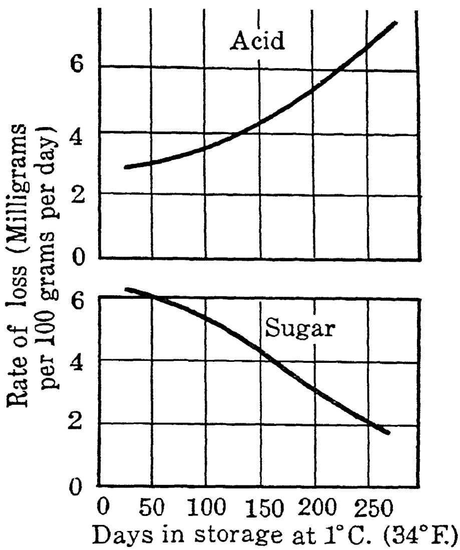


FIG. 108.—Curves indicating the rate of loss of sugar and rate of loss of acid in storage at 1° C. (34° F.) of samples of apples showing that the rate of sugar consumption decreases, while that of acid consumption increases during the development of internal breakdown.

In the respiration of succulents at night there is a restricted oxygen supply on account of the thick impermeable cuticle. This leads to incomplete oxidations at night. In the CACTACEÆ malic is the principal acid produced. In the CRASSULACEÆ isomalic acid is formed. In the MESEMBRYANTHEMEÆ oxalic acid is produced. This type of oxidation leads to a liberation of a considerable portion of the energy of the carbohydrates without the production of CO_2 which might be lost at night

by diffusion. In this manner there is conservation of carbon which, like the oxygen of the air, is not easily diffusible into the tissues. The plant acts as a carbon trap, conserving practically all of the carbon dioxide which it is able to absorb and not giving it up again to the atmosphere at night. It is enabled to do this by the production of non-volatile organic acids from the greater part of the hexose molecule.

The acidity of *Opuntia* at 4:15 P.M. represented 20.3 c. c. of 0.1 N KOH per 100 grams of tissue. At 8:15 the next morning the acidity represented 37.65 c. c.

In darkness the respiratory ratio $\frac{CO_2}{O_2}$ is as follows:

Opuntia	0.03	*Crassula arborescens*	0.24
Phyllocactus	0.33	*Sedum*	0.84

The ratio for *Opuntia* especially shows the complete conservation of carbon dioxide.

If the temperature is high, the oxidation may go further than to organic acids. In such succulents evidently there is some addition of oxygen, and we have to deal with a restricted oxygen supply rather than with absolutely anaërobic conditions. At 21° C. the production of CO_2 by *Optunia* in the presence of atmospheric oxygen was twice the production when the atmosphere contained no oxygen. At 30° C. the production of CO_2 was the same in the presence and in the absence of oxygen in the atmosphere. Even in darkness the organic acids can be oxidized if the temperature is high enough. When the cactus tissue is put into a higher temperature, the respiratory ratio is increased until the organic acid has been exhausted. At the lower temperature further oxidation of the organic acids is aided by free oxygen, but at the higher temperature the CO_2 production is not increased by the presence of free oxygen in the air because the nature of the respiratory process is changed. The CO_2 produced in an atmosphere of nitrogen does not arise from the breaking down of organic acids to as great extent as it does in an atmosphere containing oxygen. More alcohol is produced under the anaërobic condition. There is not as great economy of carbohydrate under the anaërobic conditions as there is under aërobic conditions. The aërobic oxidation to organic acid under restricted oxygen supply is more efficient in energy liberation than the intramolecular respiration of hexoses to alcohol and CO_2 under the anaërobic condition. The alcohol produced in anaërobic respiration has a high energy content.

The malic acid which has accumulated in *Opuntia* during the night

may be further oxidized during sunlight exposure by the following r action:

$$COOH\ CH_2CHOH\ COOH \rightarrow 2CO_2 + CH_3CH_2OH$$

malic acid ethyl alcohol

This photolytic disintegration of the malic acid produces carbon dioxi which may be assimilated in photosynthesis. The alcohol content b comes higher after light exposure. At 8:15 A.M. the alcohol content p 100 grams of *Opuntia* was .0032 gram while at 4:30 P.M. it had risen .0066 gram. This increase in alcohol content during the day is not d to anaërobic respiration, but due to the decomposition of malic acid.

XIII. *Anaërobic Phase of Respiration*

Probably all plant respiration has as a beginning a zymase ferment tion of hexoses. Subsequently under aërobic conditions the products of this action may be further oxidized to CO_2 and H_2O. Alcohol can be demonstrated in leaves and also in fruits. In potatoes and apples the alcohol content is usually small. Under abnormal physiological conditions in atmospheres deficient in oxygen there may be a considerable accumulation of alcohol and acetaldehyde (Fig. 109). This is notably the case in the physiological disturbance of Newton pippin apples, known as "brown core." The accumulation of acetaldehyde is a cause of storage scald of apples (Fig. 110).

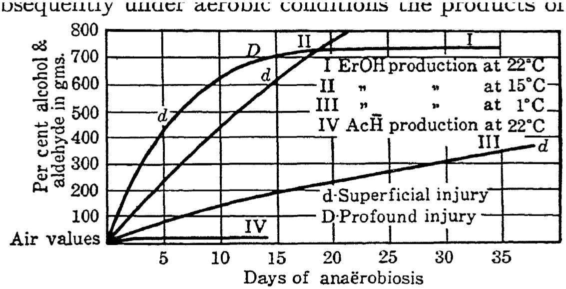


FIG. 109.—Alcohol and acetaldehyde production in apples. (After Kidd and West.)

Palladin concluded that in various leaves about one-half of the CO_2 produced was the result of zymase action. It seems probable that higher plants can oxidize alcohol. Hexose disintegration may follow several lines at the same time, the main course of the reactions being determined by the conditions of temperature, oxygen supply, and also the internal conditions, such as the acidity.

The oxygen supply is a factor of major importance in determining not only the respiratory intensity (Fig. 111) but also the nature of the oxidation processes. When apple fruits are stored in a confined space, they will consume the oxygen of the air and will produce carbon dioxide.

When the oxygen concentration has fallen to below 10% (probably at about 8% for most tissues at 46.5° F.), the nature of the respiratory process changes. The oxygen supply is insufficient to support aërobic respira-

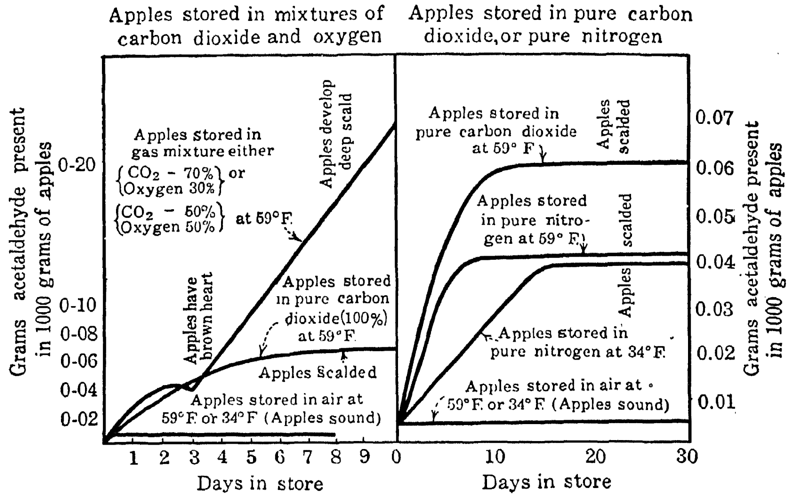


FIG. 110.—Production of acetaldehyde in apples when stored in abnormal atmospheres. (After Kidd and West.)

tion within the fruits. The temperature at which the storage room is held must be taken into account, for this changes the nature of the respiratory process, the composition of the gas in the intercellular spaces of the fruit,

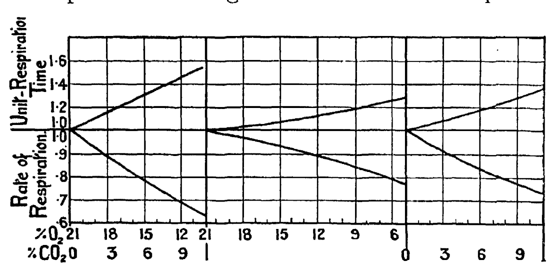


FIG. 111.—Diagram illustrating the influence of oxygen and carbon dioxide concentrations in the atmosphere upon respiration. (After Kidd and West.)

and also the solubility of CO_2, O_2, and other gases in the aqueous phases of the cells. Fig. 112 shows the relation of the temperature to the composition of the internal atmosphere of the same variety of apples (Bram-

ley's seedling) grown in different types of soils. To maintain fruits in storage as long as possible, the consumption of stored carbohydrate by respiration must be kept as low as practicable. But neither the tempera-

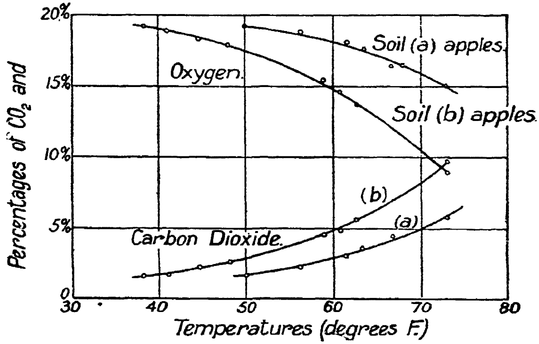


FIG. 112.—Percentages of carbon dioxide and oxygen found in the internal atmosphere of Bramley's seedling apples at different temperatures (after Ekambaram). In this figure the temperatures are the temperatures of the apple, and the gas concentrations are those present in the intercellular spaces which contain the internal atmosphere of the apple. (After Kidd and West.)

ture nor the oxygen concentration can be lowered so far as to cause the production of toxic substances. The optimum condition for the storage of apples was found by West and Kidd to be at about 46.5° F., with a

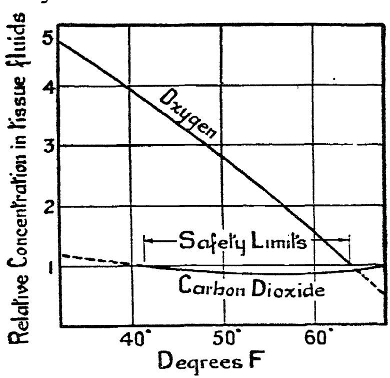


FIG. 113.—Showing the type of relationship to be expected between the temperature of storage and (1) the effective oxygen concentration in the tissue fluids, (2) the effective carbon dioxide concentration in the tissue fluids, when the external storage atmosphere contains 10 per cent CO_2 and 11 per cent O_2 in all cases. (After Kidd and West.)

concentration of oxygen of 11% and of CO_2 of 10% (Fig. 113). The CO_2 exerts a depressing effect on respiration which is greater than the effect of decreased oxygen alone. With this mixture of CO_2 and O_2 there

will be internal breakdown due to physiological disturbances when the temperature is much below about 46.5° F. and the carbon dioxide in the tissue may reach excessive concentrations. If the temperature is higher than about 46.5° F., there is a change in the nature of the respiratory process which leads to early exhaustion of the fruit. Probably the tissues do not obtain sufficient oxygen at higher temperatures. This is indicated by the rapid falling off of the effective O_2 in the tissue as the temperature increases. If the storage temperature is to be lowered, the proportion of oxygen in the external atmosphere should be decreased to prolong the storage life of the fruit. If the temperature is to be increased, the oxygen concentration should be increased above this ratio of $\frac{CO_2}{O_2}$ Fig. 114 shows the relation of the storage life to the temperature. Fig. 115 shows the length of the storage life at various concentrations of oxygen and CO_2.

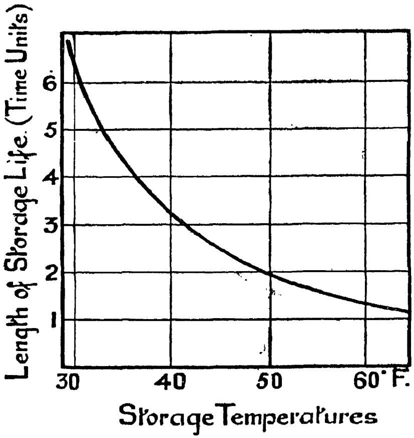


FIG. 114.—Relation between storage temperature and length of storage life. (After Kidd and West.)

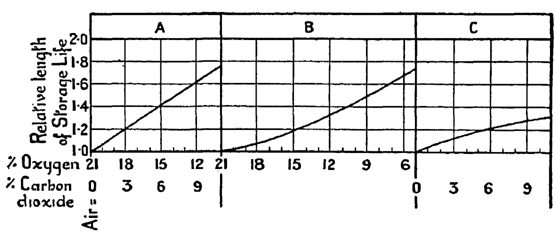


FIG. 115.—Diagrams showing the influence of oxygen and carbon dioxide concentration in the atmosphere upon the length of storage life of apples at mean temperatures. (After Kidd and West.)

XIV. *Production and Use of Oxalic Acid in Respiration*

In the respiration of higher plants oxalic acid is often an end-product. When sugar is only partly oxidized to CO_2, and partly to COOH—COOH,

the respiratory ratio is less than unity $\frac{CO_2}{O_2}<1$. This reaction may be written as follows:

$$C_6H_{12}O_6+4\ 1/2\ O_2 \rightarrow 3(COOH)_2+3H_2O+556 \text{ cal.}$$

The reaction is highly efficient in energy liberation. All evidence indicates that the source of the oxalic acid is from the incomplete oxidation of sugars. We know oxalate to be very common in plants. Calcium oxalate is highly insoluble. It is precipitated in the cells of leaves and of the wood, and in general it persists until the decay of the plant.

In corn, oxalic acid is formed when nitrates, such as KNO_3, are used as the source of nitrogen. The consumption of the nitrogen in protein synthesis leaves a base, such as potassium from KNO_3. It has been suggested that the formation of oxalic acid in this case is to regulate the oxidation. The oxalic acid serves to neutraliz. the bases liberated in metabolism, owing to the removal of the nitrate radical. No oxalic acid is produced when an ammonium salt is used as source of nitrogen. When the ammonium radical is used in protein synthesis, the anion liberated produces an acid reaction in the medium. No doubt, oxalic acid is further oxidizable by the plant and is often thus used. The oxidation of oxalic acid occurs in *Mesembryanthemum* and in fungi such as *Aspergillus*. In *Mesembryanthemum*, evidently, oxalic acid is completely oxidized to CO_2 which then is photosynthesized. In *Aspergillus* and in other fungi and bacteria oxalic acid oxidation may be the only source of energy.

Bacillus extorquens, an organism found in wood and in garden soil, uses the difficultly decomposable oxalates of calcium, barium, and magnesium. These oxalates furnish the energy necessary for its life processes. The metabolic reaction may be written as follows:

$$C_2O_4H_2+O \rightarrow 2CO_2+H_2O$$

The oxidation is accomplished by an enzyme that is similar to zymase. This enzyme can be prepared from *Bacillus extorquens*.

In the metabolism of *Aspergillus* and *Penicillium*, oxalic acid may be formed. When incomplete oxidation is induced by a lack of oxygen, or by other conditions, the oxalic acid is produced as an incomplete oxidation product of sugars. In cultures of *Aspergillus*, using ammonium salts as a nitrogen source and sugar as the carbon source, no oxalic acid is produced. If nitrates are used as the nitrogen source, oxalic acid appears. This is a duplication of the conditions for oxalic acid production by maize plants. If the carbon source is from salts of tartaric or malic acids, oxalic acid is formed. The base of these acid salts is liberated while

the tartrate or malate is used in oxidations. If free tartaric or malic acid forms the carbon source, no oxalic acid appears. Evidently oxalic acid formation is dependent upon the liberation of bases which would create an alkaline reaction in the culture medium.

In case there is plenty of base present, such as calcium, the fungus will oxidize 67% of the sugar in the medium to oxalic acid. This of course gives a very small respiratory ratio, for besides the oxygen which oxidizes the carbon atoms of sugar completely, much is used to oxidize other carbon atoms to oxalic acid which liberates no CO_2. In the process then much more oxygen is used than the volume of CO_2 produced.

FERMENTATIONS

Various fungi and bacteria which are facultative or obligate anaërobes are adapted to respiration under conditions of poor oxygen supply. The products of their respiration are in part highly reduced substances such as H_2 or butyric acid, or under more strongly oxidizing conditions alcohols or organic acids. There may be the removal of part of the oxygen contained in the hexoses or other compounds, to oxidize completely another carbon atom of the same molecule. Such intramolecular respirations are referred to as fermentations, and the living organisms which produce them are often called *ferments*. Particular organisms may be capable of producing only one type of fermentation. There is often a sequence of oxidations, requiring the action in series of various bacteria or fungi. Thus yeast (*Saccharomyces sp.*) will ferment glucose with the production of ethyl alcohol. Nearly all (95%) of the glucose may be so fermented. Upon the production of ethyl alcohol another organism may act, producing the acetic fermentation. *Mycoderma aceti* and a number of other organisms can produce this stage of the oxidation. Then the acetic acid may be acted upon by fungi (*Penicillium*) to be completely oxidized to CO_2 and water. Each step proceeds because energy is liberated in it, and such energy is available to the organisms which bring about the reactions. The ferment action is evidently highly specific, and probably is so for the reason that only certain reactions can be catalyzed by the enzymes contained in the organism which effects the oxidation.

I. *Alcoholic Fermentation*

The partial oxidation of hexoses is produced by *Saccharomyces sp.* and by certain other fungi, evidently because they cannot oxidize part of the molecule further than the alcohol stage. The reaction in the fermentation of glucose may be represented as follows:

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2CH_3CH_2OH + 27 \text{ Cal.}$$

This reaction represents almost the complete hexose metabolism of yeast. About 95% of the glucose in the culture medium may be fermented to alcohol in accordance with this reaction. About one-twentieth of the glucose may not so appear; it is either built into the compounds of the

yeast or it undergoes complete oxidation. A relatively large amount of glucose is transformed into alcohol to gain energy, since only about 4% of the available energy is liberated in the reaction.

In the fermentation of sucrose the following energy budget approximates the actual transformations occurring in fermentation by yeast (Table 29).

TABLE 29

		Heat of combustion in large cal.
Sucrose	100 gms. in nutrient medium	396.8
Produced by the fermentation:		
Ethyl alcohol	51.1 gms.	358.36
Glycerin	3.4 gms.	14.38
Succinic acid	0.65 gm.	1.99
Other compounds	1.3 gms.	5.15
CO_2	49.2 gms.	0
Heat of combustion of the fermentation products		379.88
Heat liberated 4.2% of total or		16.92

Of the energy contained in the glucose, 90% appears in the alcohol produced by the fermentation.

As transformers of materials these biological agents are as efficient as the methods of synthetic organic chemistry. Only minor quantities of other substances are formed.

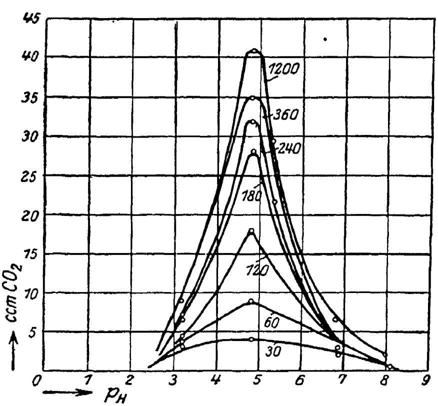


FIG. 116.—Relation of the acidity pH to the activity of zymase.

Alcoholic fermentation of glucose is produced by catalytic action. Yeast contains an enzyme, zymase, which has the ability to split glucose into alcohol and CO_2. This enzyme can act on glucose in the test-tube after the cells are killed. Zymase is most active at a pH of 4.8 (Fig. 116).

In the alcoholic fermentation probably all fermentable hexoses follow the same series of reactions. First there is enolization to a common form. They may produce then a three carbon sugar, either glyceric aldehyde or methyl glyoxal. But since it is not proved that this intermediate step occurs,

we may assume merely that a reactive isomer of the hexoses is formed. This common reactive isomer may yield pyruvic aldehyde according to the following reaction:

$$C_6H_{12}O_6-2H_2O \rightarrow \underset{\text{pyruvic aldehyde}}{2CH_3CO\ CHO}$$

The pyruvic aldehyde, being an aldehyde, can be simultaneously oxidized and reduced. One molecule may be oxidized by the Schardinger enzyme to pyruvic acid while the other is being reduced to glycerin according to the usual mechanism of the Cannizzaro reaction.

$$\begin{array}{l} C\text{—}CHO \\ \;\backslash\!\backslash O \\ CH_3\text{—}C\text{—}CHO \\ \qquad\;\;\backslash\!\backslash O \end{array} + 2H_2O \begin{array}{l} \nearrow \underset{\text{glycerin}}{CH_2OH\ CHOH\ CH_2OH} \\ \searrow \underset{\text{pyruvic acid}}{CH_3CO\ COOH} \end{array}$$

It should be noticed in this series of reactions proposed by Neuberg that the two water molecules at first removed from the hexose are again added in the simultaneous oxidation and reduction. It would seem just as reasonable not to have these definite intermediate compounds formed, but to have labile groupings. It is not possible to demonstrate pyruvic acid in the reaction, but chemists always seem to prefer the idea of the formation of definite intermediate compounds which may be just as evanescent as the labile compounds. A very common reaction of such ketonic acids as pyruvic acid is to decompose with the production of CO_2 and an aldehyde having a carbon chain one atom less than the acid.

$$\underset{\text{pyruvic acid}}{CH_3CO\ COOH} \rightarrow \underset{\text{acetaldehyde}}{CH_3CHO} + CO_2$$

The acetaldehyde produced and a molecule of pyruvic aldehyde may react according to the Cannizzaro reaction, introducing the elements of water to produce ethyl alcohol and pyruvic acid.

$$\begin{array}{lll} CH_3\text{—}CO\text{—}CHO & O & \rightarrow CH_3CO\ COOH \\ & + & \\ CH_3\text{————}CHO & H_2 & \rightarrow CH_3CH_2OH \end{array}$$

By the introduction of sodium sulphite, Na_2SO_3, into the culture medium, the acetaldehyde may be removed as an aldehyde sodium bisulphite compound in ester-like linkage. The result is that the hydrogen cannot be taken on by the acetaldehyde to form ethyl alcohol, whose formation thereby is prevented. The hydrogen then may go to reduce glyceric aldehyde to glycerin, which accumulates in abnormal amounts. The reaction then proceeds as follows:

$$C_6H_{12}O_6 \rightarrow CH_3CHO + CO_2 \cdot$$

One molecule of glycerin is produced for each molecule of acetaldehyde bound to the sodium bisulphite. Theoretically then, according to this reaction, 24% of the hexose can be converted into acetaldehyde and 51% into glycerin. In cultures not more than 36% of glycerin is so produced however. Neuberg explains this on the basis that even in the presence of sulphite part of the acetaldehyde is reduced to alcohol by hydrogen.

In *fermentation at alkaline reactions* in the presence of carbonates there are produced quantities of acetic acid. This reaction may be represented as follows:

$$2C_6H_{12}O_6 + H_2O \rightarrow CH_3COOH + C_2H_5OH + 2CO_2 + 2C_3H_8O_3$$

All three of these types of fermentation may proceed at the same time. The path which will be followed is determined by the conditions under which the fermentation proceeds.

Yeast is not marked either in its aërobic or anaërobic requirements. It evidently grows best in decreased oxidizing conditions, but not under completely anaërobic conditions. Under highly oxidizing conditions 60% of sugar is transformed by yeast into ethyl alcohol. But in practice the oxygen supply is decreased, and this allows the alcoholic fermentation of 90% of the sugar. Some glycerin and other alcohols are always produced. From the fermentation of 100 kilograms of sugar by *Saccharomyces ellipsoides* at 18–20° C., the following constituents were estimated:

Glycerin	2,120 gms.	Isobutyl alcohol	1.5 gms.
Succinic acid	452 gms.	N propyl alcohol	2.0 gms.
Acetic acid	205.3 gms.	Amyl alcohol	51.0 gms.
Isobutyl glycol	150.0 gms.	Caproic ether	2.0 gms.

In such fermentations the nature and quantity of the products is determined to a great extent by the temperature and oxygen supply. When the supply of nutrients for the yeast contains protein substances these may be deaminated and transformed into alcohols. A common source of amyl alcohol in the fermentation of grains is the leucine and isoleucine of the proteins.

$$\begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!>CH\ CH_2CHNH_2COOH + H_2O \rightarrow$$

$$\begin{matrix} CH_3 \\ CH_3 \end{matrix}\!\!>CH\ CH_2CH_2OH + CO_2 + NH_3$$

isoamyl alcohol

Increasing the leucine in the nutrient medium increases the amount of amyl alcohol produced. The substitution of ammonia as a nitrogen source decreases the yield of amyl alcohol but does not entirely prevent it, probably for the reason that yeast produces leucine from the ammonia and then transforms the leucine into amyl alcohol.

Nearly all amino acids can be so transformed in fermentations, and they are frequently the source of higher alcohols in fermentations. Succinic acid may be produced from glutamic acid.

The production of alcohol by fermentation is shown by a great many fungi. The stage to which oxidation proceeds evidently depends upon the oxidation potential and upon the organism. *Mucor stolonifera* carries on normal respiration in air, but produces alcoholic fermentation under oxygen deficiency. *Aspergillus* can produce alcoholic fermentation under reduced oxygen pressure. *Mucor racemosus* and *Macor javinicus* carry on alcoholic fermentation under all conditions; even in full aëration 80% of the sugar is transformed into alcohol.

II. *Acetic Fermentation*

The oxidation of alcohol to acetic acid is effected by a number of bacteria. The acetic acid bacteria are divided into, (1) haplotrophic bacteria, which obtain their energy from the oxidation of alcohol alone, and (2) symplotrophic bacteria, which require also organic compounds. These organisms require an abundant supply of oxygen for their action. The bacteria are very resistant to the action of the acetic acid. Evidently the oxidation of alcohol to acetic acid is accomplished by a series of enzymes similar in action to the zymase of yeast. The enzymes can be prepared from the organisms and used to produce the oxidation *in vitro*. Their reaction may be represented as follows:

$$CH_3CH_2OH + O_2 = CH_3COOH + H_2O + 112 \text{ cal.}$$

There may be an intermediate stage in this reaction in which acetaldehyde is first formed, which may be introduced into the carbon compounds of the organism. Some acetic acid bacteria can use the acetic acid as a source of energy, completely oxidizing it to CO_2 and H_2O. In the complete oxidation of alcohol through the acetic acid stage there is liberation o 326 calories per gram-molecule.

$$C_2H_5OH + 3O_2 \rightarrow 2CO + 3H_2O + 326 \text{ cal.}$$

The mixture of bacteria usually present in the mother of vinegar can produce both the alcoholic and the acetic fermentations.

The action of the acetic acid bacteria is not limited to ethyl alcohol. They also oxidize higher alcohols to the corresponding aldehydes and

acids. Propyl alcohol can be oxidized to propionic acid and butyl alcohol to butyric acid. Some of them oxidize mannitol to levulose, sorbitol to sorbose, and glucose to gluconic acid.

III. *Butyric and Lactic Fermentations*

Under anaërobic conditions a number of bacteria cause the intramolecular oxidation of carbohydrate and protein substances with the production of lactic, butyric, and other acids, and, simultaneously with these acids, reduced substances such as butyl alcohol, amines, hydrogen sulphide, mercaptans, H_2, etc. These organisms are enormously variable in their actions and represent a large group of bacteria whose principal similarity is their operation at low oxidation potential. They are either facultative or obligate anaërobes.

Of considerable interest on account of its connection with stages of aërobic respiration is the lactic fermentation. *Bacillus lactis acidi* decomposes galactose into lactic acid, reducing the alcoholic groups of the sugar to form the carboxyl groups.

$$C_6H_{12}O_6 \rightarrow 2C_3H_6O_3 + 28 \text{ cal.}$$

There is no addition of oxygen required. The energy liberation in such reactions is very little, yet this is the only source of energy for the organism.

Under anaërobic conditions *Bacillus coli* can decompose glucose with the production of lactic and pyruvic acids. The formation of lactic acid from one-half of the glucose molecule liberates more energy than is required for the production of pyruvic acid by an endothermic reaction from the other half of the glucose molecule, with the liberation of hydrogen.

$$C_6H_{12}O_6 \rightarrow CH_3\ CHOH\ COOH + CH_3\ CO\ COOH + H_2$$

In cultures evidently this reaction is not followed, probably due to interaction of pyruvic acid with the hydrogen, to produce acetic acid, alcohol, and CO_2.

The butyric fermentation of glucose by bacteria of the *Bacillus amylobacter* group occurs only under the absolute absence of free oxygen. Free hydrogen and carbon dioxide are produced as gaseous products. The reaction may be represented as follows:

$$C_6H_{12}O_6 \rightarrow C_4H_8O_2 + 2H_2 + 2CO_2 + 15 \text{ cal.}$$

In this fermentation only about 2% of the total energy of the glucose is liberated. The butyric fermentation of lactic acid should yield a still lower percentage of energy to the organism.

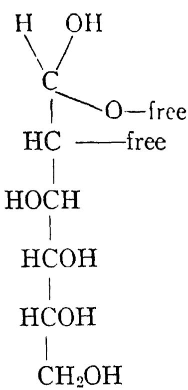
$$2H_2 + 2CO_2 -$$

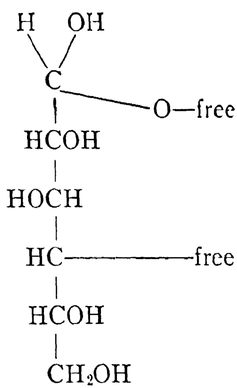
CHAPTER XXVIII

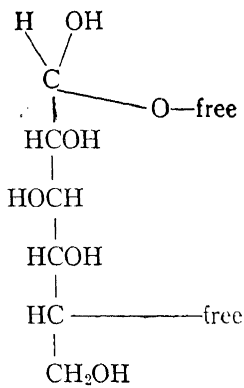
TRANSFORMATIONS PRECEDING RESPIRATION

The first step in respiration is a transformation of glucose into a more reactive isomeric form. This reactive form may be the intermediate concerned in the condensation of glucose to glycogen in fungi, and in the formation of hexose phosphoric acid. Probably the function of insulin in the animal body is to bring about this transformation. It has been suggested that the probable change is the conversion of the α- or β-forms of glucose with the butylene oxide or gamma lactone formula into the ethylene oxide form or γ-sugar, which can be normally metabolized by diabetics.

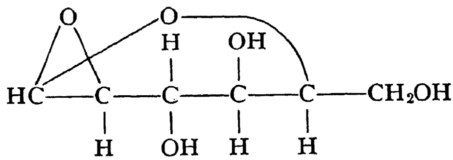
From the reactivity of the various ring structures in glucose and related compounds, Levene suggests that the most reactive configurations of glucose are those with free valences, the most reactive being the one represented by Formula I. This may be formed from the ethylene oxide ring formula. The structures represented by Formulæ II and III show a decreasing reactivity as the distance between the unsaturated bonds increases. These structures may be formed from the butylene and amylene oxide ring structures.


I


II


III

It has been shown that α-glucosan which contains an ethylene oxide ring, ferments at a higher velocity than ordinary glucose.



α-glucosan may form free valences on both the first and second carbon atoms and should be highly reactive. It may condense into polymers by opening these linkages.

The enolized sugar may break into highly reactive three carbon chains, which under conditions of poor oxygen supply yield lactic acid. The conversion of glucose to lactic acid does occur, and lactic acid may then be said to be an intermediate compound in glucose oxidation, but it may not be the normal product when abundant oxygen is supplied. The first enolization reactions are transformations within the glucose molecule and they involve little energy. However, they may be of fundamental importance in glucose metabolism. The transformations from glycogen or active glucose to lactic acid are probably also easily reversible since they involve little energy change. The cleavage of the six carbon chain into two three carbon chains does not appear to require much energy. The liberation of energy in the transformation from glucose to lactic acid is about 190 calories per gram of lactic acid which is about one-twentieth of the energy value of an equivalent quantity of glucose. In the third group of changes the oxidation of the three carbon chains formed by the dissociation of glucose involves the principal energy liberation. This involves the oxidation not of the lactic acid, which is produced only under anaërobic conditions, but of some precursor of it. These reactions are not easily reversible since they involve great energy changes.

α-d-glucose, with the gamma lactone ring, is the predominating form in crystalline glucose. It goes over in aqueous solution into the β-d-glucose until equilibrium is established between the forms. The rotation at first strongly to the right (+110°) gradually falls to an equilibrium of forms with a specific rotation of +52.5°. The conversion of the α-d-glucose form into the β-d-glucose form occurs through the intermediate stage of aldehyde structure of the Fischer formula, with a breaking of the γ lactone ring. The form of glucose represented by the ethylene oxide ring structure or formed from it and referred to as γ-glucose is the most reactive form in which glucose exists. This form readily undergoes condensation and is easily oxidized. Before the glucose is used probably the α- and β-butylene oxide forms are first converted into the γ-glucose with the ethylene oxide ring. This transformation seems to be brought about

normally by insulin. Glucose and fructose seem both to be converted into the same form, γ-glucose. The peculiar tendency of γ-glucose to condense probably accounts for the formation of its polymer, glycogen, in the fungi.

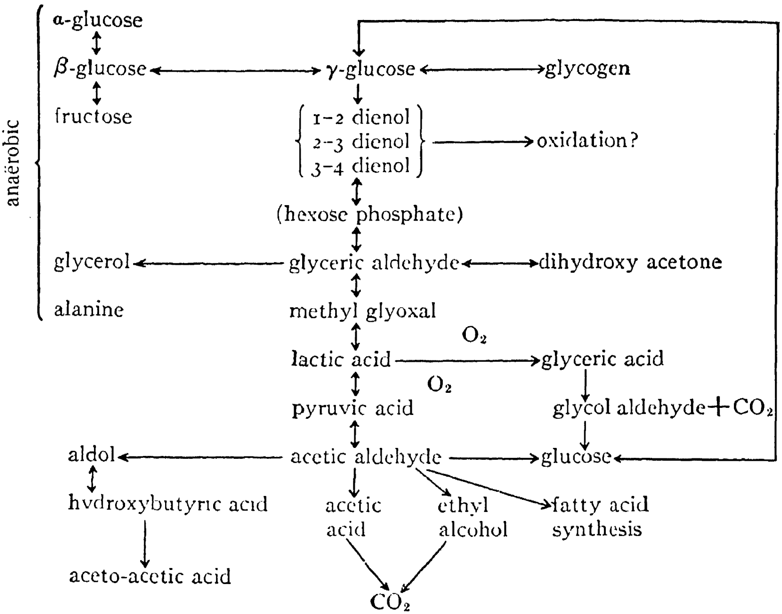
In exceedingly low concentrations of alkali the only action is the rapid transformation of α- into β-d-glucose. This is indicated by the quick change in optical rotation, with the elimination of a gradual drift of rotation. In weak alkali six isomers are formed in the d-glucose series including d-glucose, d-mannose, and d-fructose which result from the formation of 1–2 and 2–3 d-glucose dienols. The H and OH groups of the second and third carbon atoms are rendered mobile by the ionization produced by the dilute alkali (n/20 NaOH). No cleavage of the carbon chain occurs with dilute alkalies; but with strong alkali, all the rearrangements of the first three carbons may be produced, and in addition very extensive disruption of the molecule may occur. Cleavage may occur between any two carbons of the chain, but the cleavage at the center of the chain predominates. The splitting off of a single carbon atom is unusual unless oxidation occurs simultaneously. The scissive products of the dienols are exceedingly reactive. They undergo internal rearrangements, or they may polymerize to resin-like substances in the absence of free oxygen or of oxidizing substances. But in the presence of oxygen these products are generally oxidized, and no saccharinic acids or resins then are formed. The cleavage at the center of the chain with subsequent internal oxidation and reduction by the Cannizzaro rearrangement catalyzed by oxydoreductase produces lactic acid as the principal product of the anaërobic catabolism of glucose. This reaction occurs both in cells and *in vitro*. The maximum yield of lactic acid may correspond to slightly less than one molecule for each molecule of glucose. The remainder of the acids formed are formic acid, carbonic acid, and other acids which may be volatile and non-volatile saccharinic acids. This suggests that only one-half of the glucose molecule is transformed into lactic acid by alkali. In the muscle, Hill showed that a similar process was followed in respiration. However, in the cells of fungi all of the glucose may be converted into lactic acid.

In an abundant supply of oxygen the oxidation of glucose usually does not yield lactic acid in higher plants. In fact, lactic acid does not appear to be an intermediate product in higher plants, but oxidation occurs before the lactic acid stage is reached. Under aërobic conditions the oxidation of glucose seems to take place before the formation in quantity of either lactic acid, methyl glyoxal, or glyceric aldehyde.

α-d-glucose itself is not directly oxidized, so the reactive form appears to be an intermediate product between α-d-glucose and a compound as

simple as glyceric aldehyde. The intermediate products here may be any of the enols but more probably is γ-glucose.

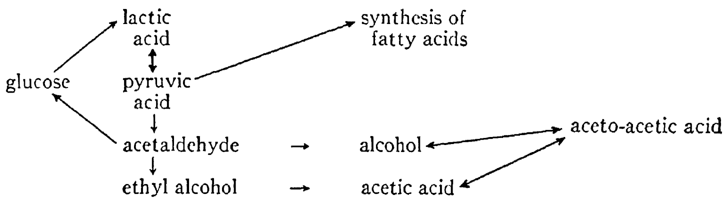
A scheme for the transformations in oxidation is given by Shaffer as follows:



An alkaline reaction favors lactic acid formation while an acid reaction seems to hinder or limit it. In the muscle, lactic acid is re-formed into glucose. In this case as well as in plants, lactic acid may yield glyceric acid, β-hydroxypyruvic acid, or glyceric aldehyde, the latter condensing to a molecule of glucose. Or, acetaldehyde may be formed with subsequent condensation and oxidation to glucose.

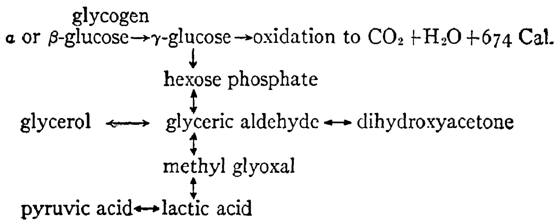
Evidence has accumulated that pyruvic acid and acetaldehyde are important intermediate compounds in the oxidation of glucose and in the oxidative re-formation of glucose from lactic acid. These compounds may serve also as intermediates when glucose passes to fatty acid or to aceto-acetic acid or vice versa.

Yeasts decompose pyruvic acid to acetaldehyde and CO_2. The reactions may be represented as follows:



The repeated condensation of pyruvic acid with loss of CO_2 and reduction may result in straight chain fatty acids with an even number of carbons.

A series of interactions producing different products under different conditions may be represented as follows:



CHAPTER XXIX

OXIDATION AND REDUCTION

I. *Oxidation-Reduction Potential*

Respiration is the process of oxidation of elaborated compounds of the protoplasm with the releasal of energy. The oxidation may proceed in more than a single step, and the products of respiration may be other than those produced in complete oxidation. In respiration we are concerned with all processes which lead to energy releasal. It is not necessary that there shall be an introduction of oxygen into combination with the substances undergoing oxidation. In fact, it would be more clear if another word such as *electronation* could be adopted rather than the term *oxidation* with its suggestion of union with oxygen. In many stages of respiration no oxygen is introduced into the system. There may be merely a shift of electrons between molecules or between parts of molecules. Oxidation is always accompanied by reduction, for oxidation is merely the giving up of negative electrons, with the liberation of definite quanta of energy. The substance which receives the negative electron is said to be *reduced* or *electronated.* In oxidations in which atmospheric oxygen is combined with substances with the liberation of energy, the molecular oxygen is the substance which is reduced. It is just as proper to call the process *reduction* as to call it *oxidation,* for one atom is always reduced when another is oxidized.

The stage to which the oxidation of a carbohydrate, for instance, will proceed will depend upon the energy which can be liberated in the process. If the conditions are such that only rearrangement of the atoms in the molecule can occur, only small amounts of energy will be liberated. In the formation of lactic acid from glucose, owing to the rearrangement and the formation of new groups, one atom takes on negative electrons from another, and the new compound produced by the reaction contains less energy than the original substance. The energy liberated may be first electronic, that is, chemical energy, involving valence electrons with releasal of proper quanta of energy. The energy may be transformed into heat, electrical energy, light, or the kinetic energy of translation of cell masses in the activities of the protoplast, but finally it all may go to produce heat. The whole process of photosynthesis results in the transformation of light energy into stored chemical energy. The process of respiration transforms this energy in one or more stages, finally producing heat.

The oxidation of a substance can be illustrated most simply by the change of charge on a single element. Iron commonly exists in solution in two conditions of electronation, namely, as ferrous ion and as ferric ion. Ferric ion, Fe^{+++}, may be reduced to ferrous ion, Fe^{++}, by addition of an electron, e, with accompanying storage of a proper quantum of energy.

$$Fe^{+++} + e \rightleftharpoons Fe^{++}$$

When the electron is taken on, the ferric ion is reduced to ferrous ion, or vice versa, and always the same quantity of energy is absorbed or liberated. The electron may again be transferred to another atom upon the oxidation of ferrous ion to ferric ion. A quantum of energy is involved in the reaction, which corresponds to the energy given up or absorbed in the change of position of the electron in the atom. That the energy so involved is usable by plants is shown by the fact that certain bacteria use this process of energy releasal from the oxidation of ferrous ion as the sole source of their energy.

Since we always have to do in respiration with simultaneous oxidation and reduction, it is not surprising to find that both the substance oxidized (oxygen acceptor) and the substance reduced (hydrogen acceptor) may be organic substances as well as inorganic ions. One molecule of an aldehyde, for instance, may be oxidized with an energy transfer to another molecule of the same substance which is reduced. Thus, acetaldehyde may be simultaneously oxidized and reduced in the Cannizzaro reaction to acetic acid and alcohol.

$$2CH_3CHO \rightarrow CH_3COOH + CH_3CH_2OH$$

There is merely an electronic transfer between the two molecules, and there is little or no energy liberated as heat; the energy transferred remains as chemical energy. No energy need be added from outside, and the reaction will proceed until equilibrium is established between the three substances, acetaldehyde, acetic acid, and ethyl alcohol. A catalyst may speed up the establishment of this equilibrium.

II. *Action of Catalysts in Oxidation-Reduction*

If ferric ion should be introduced into this system, it might take up the energy liberated from the oxidation of acetaldehyde to acetic acid and be reduced to ferrous ion. The iron then in the ferrous condition may give up this energy and the electron to reduce a second molecule of acetaldehyde to alcohol, and at the same time be oxidized to the ferric condition. In this manner the iron may act as a carrier of energy. Its presence in the system may speed up the establishment of equilibrium.

The iron is said to act in this case as an *oxidation-reduction catalyst.* This is evidently a common rôle of iron in plants. The condition for positive catalysis of the reaction is evidently determined by the ease with which the electronic transfer can be effected. If the electronic transfer can be made by iron catalysis through chemical reactions of lower order (first or second order reactions) than it can be made through the interaction of the molecules of aldehyde, the rate of the reaction will be increased, and the time to establish equilibrium will be decreased accordingly.

III. *Respiratory Chromogens*

Substances, both organic and inorganic, other than iron have the ability to serve as catalysts of the oxidation-reduction process. Probably their mechanism is similar to iron catalysis. To the whole group of complex organic substances so acting in plant cells Palladin gave the name *respiratory chromogens,* without designating the definite chemical substances concerned in every case. We may refer to them by this name properly because there are in cells certain colorless substances which on oxidation produce colored substances. The substances in plants which serve as respiratory chromogens may be formed from so-called *prochromogens,* which may be merely the combination of aromatic substances as glucosides. The glucosides may be decomposed by emulsin with a liberation of the chromogen. On oxidation of the chromogen a colored substance may be produced which may be called a *respiratory pigment.* The formation of the respiratory pigment is accompanied by an increase in the respiratory activity in the plant. This indicates that these substances may function in the respiratory process regularly. The respiratory chromogens are evidently diffusible in the tissues. When leaves of *Bryophyllum* are frozen in spots but not over the whole area, there is an accumulation of the respiratory chromogens in the frozen spots, and on oxidation these cause the frozen cells to turn dark brown or black in color. Respiration in these areas is increased. That accumulation of the respiratory chromogen has occurred in the frozen spots can be shown by subsequently freezing the whole leaf, when it may be observed that the spots first frozen remain darkest in color.

When the respiratory chromogens are extracted from plant tissues and brought into alkaline solution, they absorb oxygen very actively. We may assume that the respiratory chromogens are easily oxidizable substances which take on the oxygen of the air to form organic peroxides which again may give up oxygen to other substances. They may thus act in a manner similar to iron, as carriers of oxygen.

The respiratory chromogens are probably widely different in various

plants. There may be several different substances which function in this manner in the same tissue. The chromogens produce various colored pigments on oxidation The potato turns brown and then purplish black on oxidation. The apple turns brown; the indigo plant turns blue. The great variety of colors produced indicates differences in the substances which are oxidized to form the pigments. The chromogen of potato can be prepared in the unoxidized condition by dropping slices of potato into boiling alcohol. In similar manner colorless chromogens may be prepared from other plant tissues. The hot alcohol stops the action of oxidizing enzymes in the tissue which lead to the oxidation of the chromogens. From those chromogens which have been prepared, it seems that they are generally aromatic substances, frequently having the structure of orthodioxybenzene (quinone). To this nucleus may be joined various groups. Probably a great many substances containing a quinone ring or capable of forming it, may act as respiratory chromogens.

The substances which are easily oxidized by the atmospheric oxygen are oxygen acceptors. When the chromogen is exposed to the air, it takes on oxygen and forms the respiratory pigment which has the nature of an organic peroxide. It may again give up oxygen, that is, it then may be an oxygen donator. Since the oxygen which may be given up may combine with hydrogen to produce water, the oxygen donator may also furnish a mechanism for the removal of hydrogen from compounds. The organic peroxide is also a hydrogen acceptor. In similar manner the compound which gives up hydrogen may be called the *hydrogen donator*. Since in all cases we are concerned with the giving up or taking on of electrons, we may say that we have to deal with two classes of substances, reductants and oxidants. The reductant can accept oxygen or donate hydrogen; the oxidant can accept hydrogen or donate oxygen. Both processes are capable of performance by one substance on account of the formation or decomposition of water, which can occur on account of the universal presence of water in biological media.

The presence in animals of iron and copper containing pigments, such as hemoglobin and hemacyanin, which serve as oxygen carriers, has been known for a long time. There is present in both plants and animals also another important respiratory pigment, cytochrom, which is probably related to both hematin and chlorophyl in chemical structure. Cytochrom is a definite chemical substance which shows a spectral absorption similar to that of hematin. From its reactions it seems to be a protoporphyrin or koproporphyrin. It shows prominent absorption bands at 604, 564, 550, and 520 $\mu\mu$. Owing to the fact that it is easily destroyed by oxidation, its presence in cells is best demonstrated spectroscopically. Its quantitative distribution in tissues is closely paralleled

by the intensity of the oxygen respiration. In facultative anaërobic fungi, such as *Aspergillus oryzæ*, when the cultures are aërobic, cytochrom is present. If oxygen is excluded, the cytochrom disappears within a few hours as the fungus begins anaërobiosis. Then upon exposure again to free oxygen, cytochrom begins to form.

Cytochrom can be oxidized and reduced, the reaction being easily reversible. The oxidation and reduction of cytochrom can be observed spectroscopically from the shift of position of the absorption bands.

The function of cytochrom may be to act as an hydrogen acceptor, removing the hydrogen from water or from reduced substances, thereby starting the oxidation process. Perhaps dehydrogenation is a preliminary step in the oxidation of both sugars and certain organic acids, such as citric acid.

There is present also in both plants and animals the amino acid, cysteine, containing a sulphhydryl —S—H group. On solution of cysteine in water, and in the presence of oxygen, the sulphhydryl group may be oxidized, with the production of cystine. This same reaction is shown by glutathione or other substances containing the thione group.

$$4RSH + O_2 \rightarrow 2(RS)_2 + 2H_2O$$

At first it was thought that cysteine and thiones were auto-oxidizable substances, but Warburg has shown that traces of adsorbed iron are of the greatest importance in causing their oxidation. These several indications make apparent the important rôle of iron catalysis in respiratory processes.

Warburg explains the effect of cyanides in repressing respiration, on the basis of their effect on iron catalysis. The cyanides form irreversible combinations with the catalytic iron either of the hematin or the iron adsorbed on glutathione, and prevent its action as a catalyst. In similar manner the action of carbon monoxide can be explained, the relation between CO and the respiratory chromogen being similar to that with hemoglobin. The CO interferes with the oxygen-carrying capacity of the respiratory pigment. Cytochrom does not react with carbon monoxide. Cytochrom is not auto-oxidizable, and the effect of CO in inhibiting its action is through the removal of oxygen activated in the presence of iron. Molecular oxygen does not oxidize cytochrom. There is present in bacteria and fungi a system which binds CO loosely, much as in the blood of animals in cases of carbon monoxide poisoning. The system which takes on CO also serves to regulate the oxidation potential independent of the external oxygen pressure. In yeast, aërobic respiration is dependent upon a certain height of the oxidation potential, which is established by

this regulating system through its poising action. When the oxidation potential is below the limiting value, only fermentation takes place.

IV. *Poising Action in Oxidation-Reduction*

When a single substance is being reduced, it will take on electrons until all of the substance has been transformed into the fully reduced condition. During this time the oxidation-reduction potential changes from the value for the fully oxidized substance to that of the fully reduced substance. There is then a range of change of the oxidation-reduction potential. The shift of the oxidation-reduction potential corresponds to the shift of the pH of a medium during acid or base titration. The ability of a substance to maintain a certain oxidation-reduction potential may be called *poising action.* Poising action in reduction corresponds to the buffer effect of substances on the actual acidity, pH, of solutions. If more than two substances capable of being reduced are present in the solution, we may titrate them separately if their ranges of change in oxidation-reduction potential are sufficiently far apart, in the same manner that we may titrate 1, 2, or 3 hydrogens from phosphoric acid by the acidimetric methods. Oxidation-reduction potential will change in a manner similar to the change of hydrogen-ion concentration in acidity titrations with the hydrogen electrode in the measurement of the actual acidity (pH) of solutions.

V. *Range of Oxidation-Reduction Potentials*

In oxidation-reduction reactions in plants, we are concerned with oxidation-reduction potentials ranging all the way from the extreme oxidizing conditions existing in photosynthesizing leaves which evolve oxygen against the atmospheric pressure, to the highly reducing conditions in fermentations, at the other extreme, which liberate molecular hydrogen against the atmospheric pressure or at greater pressures. We may take the range of oxidation-reduction potentials as lying between the limits of equilibrium of the solution with one atmosphere of oxygen, and with one atmosphere of hydrogen, as the conditions commonly existing in plants.

VI. *Measurement of Oxidation-Reduction Potential*

In the reaction in which ferric ion takes on an electron and is reduced to ferrous ion, $Fe^{+++} + e \leftrightarrows Fe^{++}$, the oxidation-reduction potential can range between the value at which all of the iron is in the ferric condition, and the value at which all of the iron is in the ferrous condition. Between these two limiting values for this system, the oxidation-reduction

potential will depend upon the relative molar concentrations of the two constituents, Fe^{+++} and Fe^{++}. A platinum wire placed into the solution will take on a potential which is a function of the ratio of ferrous to ferric ion. The oxidation-reduction potential can be found as follows: If E_h is the observed potential difference between the platinum electrode and the standard normal hydrogen electrode, E_o is a constant characteristic of this particular oxidation-reduction equilibrium and equal to E_h when the ratio of ferrous ion to ferric ion $\left(\frac{\text{Ferro}}{\text{Ferri}}\right)$ is unity, R is the gas constant, T the absolute temperature, N the number of charges concerned, F the farad (96,500 coulombs), and Ferro and Ferri represent the molar concentrations of the ferrous and ferric ions respectively.

$$E_h = E_o - \frac{RT}{NF} \ln \frac{\text{Ferro}Fe^{++}}{\text{Ferri}Fe^{+++}}$$

Since substances other than ferric and ferrous ion are concerned in oxidation-reduction potentials, we may substitute the general equation

$$E_h = E_o - \frac{RT}{NF} \ln \frac{[\text{Red}]}{[\text{Ox}]}$$

in which Red and Ox refer to the molar concentrations of reductant and oxidant.

In a solution in which the oxidation-reduction potential was great enough to evolve hydrogen, a platinum electrode surface will become saturated with hydrogen. It will act as a hydrogen electrode. The hydrogen adsorbed upon the electrode is in equilibrium with the hydrogen ion, H^+, in the medium and with hydrogen in the atomic condition. The pressure of hydrogen at the electrode P, or the pressure under which it is evolved, will be a function of the H^+ and H concentrations in the medium. The components of the oxidation-reduction medium may then interact either with hydrogen ions or water, liberating gaseous hydrogen and building up on the electrode a definite pressure of hydrogen, or, vice versa, the hydrogen on the electrode may go into solution in the medium to cause the reduction of the substances in the system. When a constant pressure of H_2 is maintained at the electrode in equilibrium with the solution, at a known pH, the potential at the electrode may be used to measure the oxidation-reduction potential because it refers to the potential produced by a definite pressure of hydrogen.

$$E_h = EH - \frac{RT}{NF} \ln \frac{\sqrt{P}}{[H^+]}$$

We may calculate the oxidation-reduction potential of solutions as follows (Clark): Given an equimolecular mixture of ferrous and ferric chlorides in solution at pH 1. A platinum electrode in such a solution will have a potential about 0.75 volt more positive than the n/1 hydrogen electrode. If this potential is taken as the difference between the n/1 hydrogen electrode and a hydrogen electrode at pH 1, we may calculate the pressure of H_2 in equilibrium at the second electrode at 25° C. $\frac{RT}{F} = 0.059$ from the equation:

$$0.75\ \text{v} = -0.059 \log \frac{\sqrt{P}}{0.1}$$

$P = 10^{-27}$ atmospheres pressure of H_2 in equilibrium with the second electrode. In similar manner, using an oxygen electrode, the equilibrium pressure of O_2 could be determined. Fig. 117 gives the theoretical relationships between the partial pressures of oxygen and hydrogen, the electrode potential, and the pH of solutions.

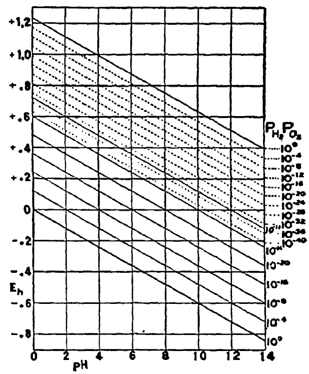


FIG. 117.—Theoretical relations between electrode potential Eh, pH, and partial pressures of hydrogen and oxygen. Each decrement of the partial pressure of hydrogen by 10^{-4} shifts the potential of a hydrogen electrode at 30° C. $+0.03 \times 4 = 0.12$ volt. Each decrement of the partial pressure of oxygen by 10^{-4} shifts the theoretical potential of an oxygen electrode—$0.015 \times 4 = 0.06$ volt. (After Clark.)

Since it is more convenient to use indicators which show a color change than to measure the actual potential differences, Clark has devised a series of oxidation-reduction indicators. These may be used at known pH to determine the oxidation-reduction potential.

The actual conditions of oxidation within cells have been but little studied. Yet there are evidently greater fluctuations of the oxidation-reduction potential normally occurring in plants than in higher animals in which the oxidation-reduction potential of the blood is regulated. Plants show great fluctuations in the oxidation-reduction conditions. Perhaps this is related to the greater range of oxidations and reductions which can be effected by plants. When closed off in a confined space, plants will absorb quantitatively the oxygen present without death occurring. A higher animal requires an oxygen pressure almost equal to that present in the normal atmosphere for its existence. The respiratory pigments of plants evidently can take in oxygen under more reducing conditions than can the hemoglobin of the blood.

CHAPTER XXX

RESPIRATORY ENZYMES

The respiratory process is catalytic, and of the catalysts which take part in the process there are some whose action is destroyed by heating and by other agencies. These thermolabile substances have usually been referred to as *enzymes*. By various reactions there have been separated several different types of action, although all of these substances are really oxydoreductases, that is, they catalyze the simultaneous oxidation and reduction of substances. The functions and identities of these substances have never been sufficiently studied. Some which were formerly thought to be single enzymes have been shown to have several constituents acting together.

We may differentiate the following types of action among the thermolabile catalysts which seem to be concerned with respiration.

1. Catalase is the enzyme which hastens the cleavage of hydrogen peroxide into water and molecular oxygen. About the only reason we have for considering this enzyme to be of importance in respiration is the fact that it is almost universally present in living things. Its action in living organisms would depend upon the presence of hydrogen peroxide in cells, which is by no means proved. There is no reason for assuming that the only action of this enzyme is in the cleavage of H_2O_2, yet this is taken as the measure of its activity. By its action H_2O_2 is decomposed to H_2O and O_2, with an electronic transfer from one of the oxygen atoms according to the following equation: $2H_2O_2 \rightarrow 2H_2O + O_2$. Catalase is most active in neutral solutions (Fig. 118).
2. Oxygenase is the enzyme which hastens the oxidation of substances by the oxygen of the air. By its action, molecular oxygen is reduced and enters into combination with the oxidizable substance. The thermolability is about the only reason for assuming that this is an enzyme at all, and that is no very good reason. A great many known compounds may be oxygen carriers and may be destroyed by heat.
3. Oxydoreductase, or reductase, is the enzyme which causes the simultaneous oxidation and reduction of substances, in which a transfer of oxygen from one molecule to another is required. A typical action of this enzyme is the simultaneous reduction of nitrate to nitrite and oxidation of benzaldehyde to benzoic acid.
4. Zymase is the enzyme which initiates and hastens the decomposition of glucose into carbon dioxide and ethyl alcohol:

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH$$

5. Peroxidase is the enzyme which hastens the transfer of oxygen from an organic peroxide to an oxidizable substance. Much of the peroxidase action may be due to such thermostable substances as iron or manganese compounds. There is a question as to whether substances capable of being called peroxidase enzymes exist in plants. Possibly the change of

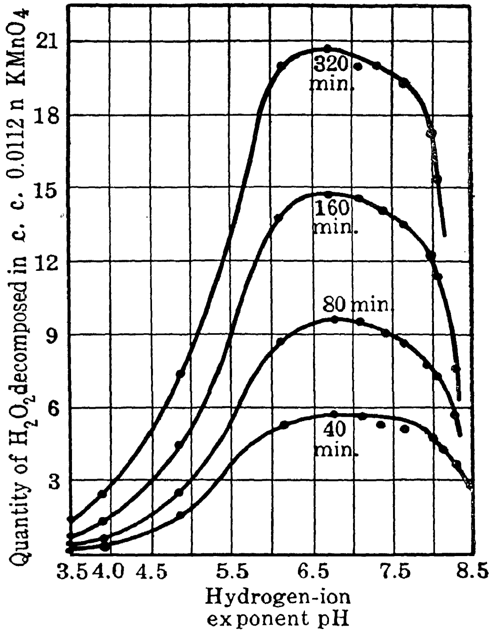


FIG. 118.—Relation of the acidity (pH) and time to the activity of catalase.

reactivity on heating shown by these substances may be due to partial inactivation of adsorbed iron or manganese compounds. If there were no change in activity with heating, we should be forced to conclude that no enzyme was concerned, or to amplify more fully, our expressed ideas as to what constitutes an enzyme. It is probably best to consider that enzyme actions may be caused by different substances rather than by one substance alone. We should more properly speak of enzymes as types of catalytic action rather than as actual substances; however, one single substance chemically identified may have the action of enzymes.

In plants which produce gluconic acid as an oxidation product there is present a glucose oxidase. This enzyme can be found in the pressed juice from cultures of *Aspergillus* and *Penicillium* which have been grown on a solution containing glucose. This enzyme will oxidize d-mannose

and d-galactose in minor degree, but will not act upon d-fructose, d-xylose, d-arabinose, acetaldehyde, dioxyacetone, glycerin, or calcium d-gluconate. It is possible that there may be in the pressed sap d-galactose and mannose oxidases.

Glucose is oxidized by glucose oxidase in the presence of oxygen to

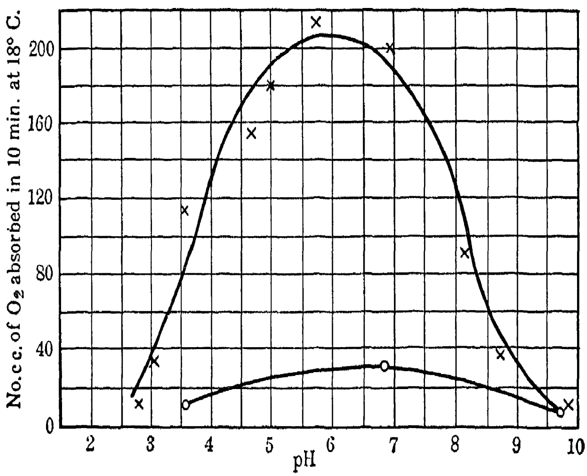


FIG. 119.—Relation of the acidity to the activity of glucose oxidase. x—x, glucose oxidase, buffer, and glucose. o—o, glucose oxidase and buffer without glucose.

gluconic acid. The enzyme will not act in the absence of oxygen; it differs from zymase in this regard and also in its inability to oxidize fructose. Phosphates are not coenzymes of glucose oxidase. The optimal acidity for glucose oxidase is 5.5–6.5, as shown in Fig. 119. With increasing the temperature up to 30° C., the activity of the enzyme increases, but there is a decrease in its activity with time, which causes the activity to decrease rapidly at the higher temperatures. The temperature of inactivation is about 73° C.

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PART VI

RESPIRATION

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